A COMPARATIVE STUDY OF AUTONOMIC FUNCTIONS AND SERUM LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN PSORIATIC SKIN AND PSORIATIC ARTHRITIS PATIENTS

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CERTIFICATE

This is to certify that the dissertation entitled "**A COMPARATIVE STUDY OF AUTONOMIC FUNCTIONS AND SERUM LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN PSORIATIC SKIN AND PSORIATIC ARTHRITIS PATIENTS"** is the bonafide original work of **Dr.I.KANAGASHREE** in partial fulfillment of the requirements for **M.D. (PHYSIOLOGY) BRANCH – V** Examination of the Tamil Nadu Dr. M.G.R. Medical University to be held in2015.

The period of study was from May 2014 to September 2014**.**

DEAN

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A comparative study of autonomic functions and serum Vascular Endothelial Growth

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INTRODUCTION

Psoriasis is a chronic systemic immune mediated inflammatory disease with a wide spectrum of clinical manifestation affecting approximately 2 – 4 percent of general population[i]. Psoriasis is a noncontagious primary dermatological disorder affecting skin and nails.

The exact cause of the disease is obscure, but genetics, immunity, environmental and psychological factors plays keen role in development and progression of the disease[ii].Though the etiology is not defined, the disease follows relapsing, exacerbation and remitting course.

It can affect any age, from infancy to old age, but the usual age of onset is between 25- 30 years. Incidence of psoriasis shows no sexual predilection, but women have earlier age of onset[iii]. Around30percent of psoriatic patients have a family history of disease, either in first or second degree relatives.

The most common skin manifestations are dry itchy erythematous plaques with asymmetrical borders, and silvery white scales due to hyper proliferation of skin[iv].The white scales are accumulation of the hyper proliferated skin cells waiting to shed and the erythema is due to increased blood

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CONTENTS

A Comparative study of Autonomic functions and serum levels of Vascular Endothelial Growth Factor in Psoriatic skin and Psoriatic arthritis patients

Aim: To access and compare the Autonomic functions (ANS) and serum levels Vascular Endothelial Growth Factor (VEGF) in Psoriatic skin (Ps) and Psoriatic Arthritis (PsA) patients.

Materials and Methods: The study contains three study groups containing 15 men and women of age between 20- 40 years with normal resting blood pressure. Those with skin lesions and no clinical evidence of arthritis comes under Ps group and those having clinical evidence of arthritis with or without skin lesions comes under PsA. All the 90 individuals are subjected to 5 minute Resting HRV and Orthostatic, Isometric Hand Grip, Cold Pressor, Deep breathing and Valsalva tests. Serum was collected and VEGF analysed using standard ELISA kit.

Results: The patient groups showed increased LF, LF/ HF ratio, serum VEGF and also elevated diastolic blood pressure in isometric hand grip, cold pressor test. The HF, 30/15, E/I and Valsalva ratio. Inter comparison between Ps and PsA is statistically significant.

Conclusion: ANS dysfunction is present in both Ps and PsA patients in form of sympathetic over activity and parasympathetic withdrawal and elevated serum VEGF levels. The ANS dysfunction is more in PsA and no significance of

serum VEGF values between the groups. VEGF value reflects the disease severity.

Key words: ANS functions, Psoriatic skin, psoriatic arthritis, VEGF, Resting HRV

INTRODUCTION

Psoriasis is a chronic systemic immune mediated inflammatory disease with a wide spectrum of clinical manifestation affecting approximately 2 – 4 percent of general population (Menter et al)[1]. Psoriasis is a noncontagious primary dermatological disorder affecting skin and nails.

The exact cause of the disease is obscure, but genetics, immunity, environmental and psychological factors plays keen role in development and progression of the disease (Visnja et al)[2]. Though the etiology is not defined, the disease follows relapsing, exacerbation and remitting course.

It can affect any age, from infancy to old age, but the usual age of onset is between 25- 30 years. Incidence of psoriasis shows no sexual predilection, but women have earlier age of onset[3]. Around30percent of psoriatic patients

have a family history of disease, either in first or second degree relatives.

The most common skin manifestations are dry itchy erythematous plaques with asymmetrical borders, and silvery white scales due to hyper proliferation of skin (Steven R et al)[4]. The white scales are accumulation of the hyper proliferated skin cells waiting to shed and the erythema is due to increased blood supply of the epidermis to support hyperproliferation. These skin lesions are raised and causes thickening of skin. The lesions of skin vary in severity from localized lesions to lesions involving entire skin. The lesions after healing causes hyperpigmentation without scar formation, because the lesions involve only the superficial layer of skin.

Almost 40-50 percent of all psoriasis patients develop distinctive nail changes. The appearance and consistency of nails are altered. These nail changes are due to inflammation of nail matrix or nail bed or involving both. The common expression of nail psoriasis is pitting of nails and distal onycholysis (Hermenio)[5].The other signs are yellow discoloration, paronychia, subungal hyperkeratosis,

and onychodystrophy. Patients with involvement of nails report to have higher prevalence of PsA. Almost 90 percent of patients with arthritis have nail involvement.

Psoriasis has a significant systemic involvement including joint and soft tissue and mucous membrane inflammation, eye, cardiovascular and intestinal problems[6,7]. Psoriasis has increased prevalence of conventional cardiovascular risk factors which not only contribute to overt disease but also to subclinical manifestations (A M Tobin)[8]. Psoriasis alone is considered as a risk factor of developing coronary heart disease and the risk increases with severity of the disease. There are variety of ocular manifestations including conjunctivitis, dry eye, episcleritis, and uveitis. The mucous membrane inflammation manifestation as painful mouth ulcers and urethritis.

The other auto immune manifestations are inflammatory bowel disease, and multiple sclerosis. Not only auto immune diseases, some lymphomas are also associated with psoriasis. The lymphomas associated with

psoriasis are cutaneous T cell lymphoma, Hodgkin's lymphoma and Non- Hodgkin's lymphoma.

Psoriatic arthritis (PsA) is a seronegative chronic inflammatory musculoskeletal disease affecting 7-20 percent of patient with skin psoriasis. Like skin disease the clinical manifestation of arthritis has wide spectrum of manifestation like monoarticular, oligoarticular, and polyarticular joint involvement. Any joint can be affected by PsA.

Apart from joint manifestation of arthritis, inflammation of tendons and ligaments is common in PsA. This inflammation is called as enthesitis and it is characteristic of PsA. The cause of PsA is same as that of skin disease but the reason for only some of the skin disease patients developing arthritis and other severe manifestations is abstruse. It is a long term manifestation affecting at any age.

In about 70 percent of PsA patients, skin manifestations develop first, and then musculoskeletal

manifestation develops after a variable duration. PsA and skin manifestation develop synchronously in 15 percent of patients. In the remaining 15 percent of PsA patients musculoskeletal manifestations develop first and skin lesions develop later in their life. The course of the disease, its response to treatment, and prognosis is highly variable.

Clinical manifestation of PsA is joint pain, swelling, inflexibility, erythema, reduced agility of involved joints. The dispersion of joints involved in PsA is frequently asymmetrical, but as the number of joints involved is amplified, there is a tendency toward symmetric involvement.

The pathogenesis is same for both the skin and joint manifestation. It is a multi-genic autoimmune disease. The common manifestation is hyper-proliferation and angiogenesis which is formation of new blood vessels from pre –existing vessels, that aids proliferation. Vascular Endothelial Growth Factor (VEGF) is a signalling protein that is one of the essential component for angiogenesis.

VEGF causes proliferation, sprouting, migration of endothelial cells (Ferrara et al)[9].

VEGF is a potential survival factor for endothelial cells in physiological and pathological conditions. VEGF has stimulatory effect of recruitment of inflammatory cells. In angiogenesis it promotes the expression of protease in the pericellular matrix degeneration. It is a permeability factor which increases the permeability of endothelial cells. It forms vacuoles, fenestrations, intracellular gaps and vesico- vascular organelles.

VEGF is elevated in site of skin lesions as well as in the inflammed synovium in psoriasis. The serum level of VEGF in serum correlates with the disease severity.

Psoriasis is a systemic disease and also involves the Autonomic Nervous System (ANS). Heart Rate Variability measurement is a non –invasive technique that assess the ANS functions. It can measure the sympatho – parasympathetic balance. The symptoms of impaired sympatho – parasympathetic balance may not be present or

non-specific in psoriasis. By eliciting ANS function tests, along with resting HRV, the presence and extent of ANS dysfunction can be found in psoriatic skin and psoriatic arthritis patients.

The early development of cardiovascular disease is psoriatic patients is well established. The reason is multiple risk factors like early atherosclerosis, metabolic syndrome, and associated ANS dysfunction. The epidermal homeostasis is tightly governed by the sympathetic nerves of skin and the sympathetic system checks the rate of proliferation and cornification of keratinocytes. In psoriasis due to unknown antigen trigger the immune cells are activated and continuously produce activated cytokines which end in hyperproliferation. The sympathetic system is over activated to control the local proliferation. Similarly in PsA the inflammation triggers sympathetic nervous system and hypothalamo pituitary adrenal axis to secrete catecholamines and corticosteroid respectively and end in ANS dysfunction.

By conducting ANS study the pathogenesis and progression of psoriasis can be understood and aids in treatment of the disease. Though psoriasis is a well-known old disease the cure for the disease is yet to be found.

REVIEW OF LITERATURE

2.1 PSORIASIS HISTORY

Since ancient times psoriasis is a well-known dermatological disease. It is mentioned as 'tzaraat' in Bible's book of Leviticus, which is believed as psoriasis by scholars. It is also mentioned that 'tzaraat' was caused by sins. Hippocrates 460 – 377 BC, described many skin diseases as lepra, leichan, alphos, psora. Psoriasis was not exactly described by him, but he included many itching and scaling skin disorders under this condition.

In India Ayurvedic medicine is being practiced since antiquity. CharakaSamitha, an ancient Ayurvedic scholar, in his book mentioned a disease 'Khusta' which is believed to be leprosy. The works of PaulRichteret,al[10].

suggested that the disease is psoriasis. It was in the first century in Roman Empire psoriasis was first described by Cornelius Celsus. He described it as scaling lesions of skin also affecting nails. Celsus used pitch and sulphur for treating it. He did not use the word psora, or psoriasis, Bechet, PE et al (1936)[11].

It was Galen who coined the word 'psora'. He described the term for seborrheic dermatitis which has itchy and scaly eruptions. Many other persons used the term psora to describe the skin disease similar to psoriasis. In middle age nothing much was known about the prevalence and treatment of psoriasis. In the eighth century, the Arabian physicians were the first to distinguish psoriasis from other scaling skin diseases and they used a kind of psychotherapy in treatment (Shafii, M.etal.1979)[12]. Psoriasis was mentioned as 'lepragrecorum' by professor Hieronymus Mercurialis in his book of skin diseases in sixteenth century.

In 1808 psoriasis was first recognized as a separate skin disease (WillanR. et al)[13]. Robert Willan (1757 – 1812) described some papulosqumous diseases. He differentiated psoriasis and leprosy, mentioned as psoraleprosa and lepragrecorum. He also noticed psoriasis more common in extensor surfaces, scalp and

affects nails. In 1841, Ferdinand Von Hebra an Austrian dermatologist, was the first to name the condition as 'Psoriasis' derived from the Greek word psora, which means itch[14.] He also described psoriasis as a separate skin disease and described its various manifestations.

In the nineteenth century varieties of psoriasis has been described, like pustulosa generalisata by Von Zumbush 1910, and psoriasis palmo- plantaris by Barber – Konigsbeck. Some of signs were also described during this period like Kobner phenomenon in 1872, by Heinrich Kobner and Heinrich Auspitz described Auspitz sign. Auspitz also introduced the pathophysiological terms like acanthoma and parakeratosis typical for psoriasis.

Micro morphology of psoriasis was described by Willam Munro, Hebra, and Unna. They described accumulation of neutrophils in stratum corneum of skin and used the term 'micro abscesses'. During early twentieth century Woronoff (1926) described Wornoff ring around psoriatic plaques. In 1927 FranjoKogoj described the spongiform pustule in pustular psoriasis. Van Scott, in 1963 demonstrated the hyper proliferation of epidermis in psoriatic skin.

2.1.2CLASSIFICATION OF PSORIASIS

There is no single classification of psoriasis that satisfies all the clinical manifestation of the disease. The criteria used are intermixed and the subclasses are non-exclusive.

According to the spectrum of clinical manifestations the following classification is commonly used.

S. No	Classification	Salient Clinical features
1	Psoriasis vulgaris	Chronic plaque like, stationary psoriasis
2	Guttate psoriasis	Small ,drop like spot lesions
3	Pustular psoriasis	Pus filled straw colored blister lesions
4	Erythrodermic psoriasis	Predominant erythema, limited scaling
5	Nail psoriasis	Characteristic nail changes

2.1.3 Pathogenesis

Skin is the first protective barrier, the largest and complex organ of body, containing neural networks, glands, connective tissues, blood vessels and immune cells. It also has homeostatic functions, modulated by local and systemic factors. Epidermal homeostasis is maintenance of its structure and the balance between proliferation and cell loss either by desquamation (skin shedding) or apoptosis (Stark HJ et al 2006)[15].

The stem cells present in the basal layer of epidermis undergoes continuous proliferation and provides new cells for renewal of cells lost by routine turn over and also during injury (Blanpain, C et al 2009)[16].The basal cells on proliferation give rise to keratinocytes (squamous epithelial cells), the young skin cells, which ascend up to superficial layer of skin the stratum corneum where they differentiate to corneocyte. Corneocytes are non-living epidermal cells which are shed by the process called as desquamation or undergo apoptosis.

Cornification is a process whereby living keratinocytes are differentiated into non-living corneocyte. Normally the keratinocytes takes fourteen days to differentiate to corneocyte, during which it ascends from the basal layer to the superficial layer the stratum corneum. A Cornified layer is present in outermost layer of epidermis, which is formed by the lipids (mainly ceramides) present in cell membrane and their covalently linked structural proteins.

Factors affecting epidermal homeostasis are

1) The Ceramides:

The lipids which are synthesized in the keratinocytes are stored in lamellar bodies and secreted as intercellular domains of the uppermost layer of epidermis. These lipids form a water impermeable barrier membrane in the skin. There are several lipids present in barrier layer of which ceramides are important and found in higher concentrations (Candi E, et al 2005)[17].These ceramides acts as signalling molecule and regulates the proliferation, differentiation and apoptosis of cells (Hannun, YA et al)[18].

2) The Proteinase:

Suprabasal keratinocytes express inactive protease (Brattsand M et al 1999)[19], of which those belong to kallikrein family of serine proteases is important. Based on their proteolytic domain, these proteases are classified into cysteine, threonine, metalloproteinase, and serine proetinase. These enzymes are activated in stratum corneum and appear in the intercellular spaces and they play a role in desquamation. The differentiation program of epithelium requires these proteases help in detachment of Corneocytes from one another without

disruption of the epithelial barrier. Serine proteases inhibitors on tropical application have shown to accelerate the barrier recovery after abrogation (Hachem JP et al 2006)[20]. Thus serine proteases cause the lysis of corneodesmosomes (Kanitakis J et al 2006)[21] and proved to play a role in epithelial barrier function.

3) Epidermal junctions and the adherent junction proteins:

Epidermal junction is the basement membrane synthesized by the dermal fibroblasts and the basal keratinocytes. It acts as a mechanical support and helps in adhesion of epidermis and dermis. It plays an important role in the regulation of exchange of metabolic products between dermis and epidermis. The keratinocytes in basal layer are adherent to one another and also to the basement membrane through gap junctions, adherent junctions out of which desmosomes is most characteristic in this layer. Maintenance of desmosomes in the basal layer is important in epidermal homeostasis.

4) Intracellular and extra cellular Calcium:

In the basal layer formation of new adherens junction and desmosomes requires efficient concentration of extracellular calcium (Hennings H et al)[22]. In cell culture medium of keratinocytes, raising the calcium concentration from 0.05

mM to 1.2 Mm stimulates the cells to form strong cell to cell adhesions. Also proper calcium concentrations, both intracellular and extracellular are essential for proper proliferation and cell differentiation of keratinocytes.

5) Secretory function of keratinocytes:

Keratinocytes produce Catecholamines locally (Christian E et al)[23]and also wide varieties of cytokines like tumor necrosis factor and IL 1α, IL 1β and IL-6 in addition to keratin protein. Any interruption in epidermal barrier causes increased secretion and expression of these cytokines. These cytokines stimulate synthesis of lipids; regulate their metabolism helps in maintaining the barrier homeostasis function of epidermis.

6) Sympathetic receptors:

Skin is another important peripheral endocrine organ that is regulated by central regulatory mechanisms. Keratinocytes express beta 2 adrenergic receptors (Tseraidis GS et al)[24]. These receptors on activation control the proliferation of keratinocytes by protein - kinase dependent pathway(Puller et al)[25]. This receptor also controls the rate of migration of keratinocytes by cAMP independent pathway. Catecholamines increase the calcium entry and aids proliferation, migration and differentiation of keratinocytes. The expression of these

receptors is more in basal layers of epidermis than in stratum corneum. They also play important role in re-epithelialization, and wound healing.

There exist two hypotheses for the pathology of psoriasis. The first hypothesis states that psoriasis as a purely skin disease involving the skin only. The disease is due to faulty hyperproliferation of epidermal cells the keratinocytes, with their incomplete differentiation. These keratinocytes also show reduced apoptosis. The support to this hypothesis is that in some patients the disease involves only the skin and do not show any systemic manifestations. This hypothesis is not accepted since it is well accepted that psoriasis is a systemic disease and patients who show initial skin manifestation later develop systemic manifestations.

The second hypothesis states psoriasis is an immune mediated disorder (Wu,JJ. et al2012)[26].The hyperproliferation is secondary to inappropriate activation of cell mediated immune system. Skin has effective immunological surveillance system. It has antigen presenting cells, T cells, tissue macrophages, granulocytes, fibroblasts, capillary endothelial cells of dermis, mast cells, cytokines synthesized locally. All the above mentioned cells together with draining nodes constitute skin a primary lymph organ.

These cells interact by cytokines in response to antigens which are presented by the antigen presenting cells.

The antigen may arise from any stimulus like infection, chemicals, irritating substances, ultraviolet rays, drugs or even stress. Normally there is controlled response, but in psoriasis the response is prolonged, uncontrolled and there is imbalanced secretion of cytokines (Krueger JG.et al)[27].

In psoriasis due to some unidentified antigen stimulation the epidermal dendritic cells the Langerhans cells, which recognizes and captures the antigen. These cells migrate to local lymph nodes and present them to the T- helper cells. In psoriasis there is immune deregulation and the functions of T cells are not regulated. Any stimulation of T cells causes a cascade of inflammatory responses in psoriasis.

The activation of T lymphocytes releases pro inflammatory cytokine Tumor Necrosis Factor- α and interleukin -2. The activated Langerhans cells release IL12. These cytokines ultimately regulate the genes that code the transcription of other cytokines like, interferon gamma, Tumor Necrosis Factor etc, which helps in

proliferation, differentiation and maturation of T cells into memory effector T cells (memory T cells to that particular antigen). These T cells migrate to the skin from the lymph node; accumulate around the dermal blood vessels. These events are a normal response of epidermis to any antigen, but in psoriasis that will be initial step in series of immunologic changes resulting in a psoriatic plaque.

The production of several cytokines around dermis and in epidermis creates a pro- inflammatory environment, where the endothelial cells of dermal vessels initiate to express adhesion molecules like Intra Cellular Adhesion Molecule – 1(CD54 or ICAM-1), E-selectin(CD62E), Vascular Cell Adhesion Molecule - 1(VACAM-1 or CD 106). The molecular alterations in the skin cause neutrophils and lymphocytes to migrate to dermis. Among the pro- inflammatory chemokines, IL 8 is related to Margination of neutrophils plaque psoriasis. These leucocytes are attracted by the inflammatory chemokines released by the keratinocytes (Salgado A et al)[28].

The Dendritic cells in skin which is CD+11c positive, produces TNFα, IL-12, IL23 and IL20.These cytokines ultimately regulate the genes that code the transcription of other cytokines

like, interferon gamma, Tumor Necrosis Factor etc, which helps in proliferation, differentiation and maturation of T cells into memory effector T cells (memory T cells to that particular antigen). These T cells migrate to the skin from the lymph node; accumulate around the dermal blood vessels.

After the T cell migration there is subsequent increase in migration of other leukocytes especially neutrophils and lymphocytes into the dermis and there is increased proliferation of keratinocytes. The hyper proliferative response of keratinocytes decreases the time of transit from base of epidermis to the superficial corneal layer. There are three phases in cytokine pathway (Kunz M et al)[29].

Phase I:

Production of Interferon Gamma by CD3 + Th 1 cells, inn response to IL12 is phase I of cytokine pathway.

Phase II:

Continuous activation of keratinocytes and formation of TGFβ in the inflammatory field is the initial step. IL12, TGF β, IL 23, induces the production of IL17, from the T cells.

Phase III:

IL 17 acts with IL 20, IL 22 and IL24 produced by activated macrophages ultimately results in enchased keratinocyte activation, proliferation which belongs to phase III of cytokine pathway. The transit time reduces from the normal 28 days to 2-4 days in psoriasis.

2.1.4 Diagnosis:

The lesions in skin in due course of disease in the same patient differ over time. There is no specific diagnostic test to predict the future psoriasis and so patients presenting first time with psoriatic lesions are diagnosed as 'latent psoriasis'[30]. Patients showing subjective variation are diagnosed as 'minimal psoriasis' due to lack of validated criteria. The signs of psoriasis are called as 'stigmata of psoriasis'.

Signs

1. Hyperkeratotic plaques on the skin surface with or without scaling.
2. In palms and soles keratolysis like lesions.
3. Severe dandruff like lesions well beyond the hair line.
4. Nail pitting

5. Multiple sterile paronychia.
6. Subungal hyperkeratosis
7. Onycholysis in the absence of fungal infection.
8. Sharply marginated penile erythema without fungal infection.
9. Well defined erythematous plaques which may be symmetrical or not in distribution.
10. Healed lesions shows hyperpigmentation and shows no scarring, since psoriasis involves only epidermis.
11. Scaling of lesions is characterized to psoriasis, but it may be absent in severe form and in acute lesions. The scales are silvery white, and vary in thickness.
12. Auspitz sign: removal of scale from lesions shows tiny bleeding points due to hyperproliferation of dermal blood vessels.
13. Koebner phenomenon: development of new lesions at site of traumatized skin (Griffiths, C et al)[31].

In most patients diagnosis is established by the clinical presentation, family history, and by showing the presence of Auspitz sign and by the history of Koebner phenomenon. Very rarely to diagnose, biopsy of skin is essential in case of psoriasis. The predominant characteristic microscopic features of psoriasis are

- Epidermal hyperplasia
- Inflammatory infiltrate
- Vascular changes

The epidermal changes are keratinocyte hyperplasia, attenuated or absent granular cell layer with poor keratinocyte maturation, differentiation (McGrath JA et al)[32] along with epidermal thickening. The epidermal projections are clubbed and interdigitate with dermis. The cells in stratum corneum also show poorly differentiated, immature cells with nucleus (Haftek M et al)[33]. Normally the corneocytes lack nucleus on maturity. All these changes in epidermis cause thick plaques with scales.

The dermis shows inflammatory infiltrate initially, and these infiltrates evade the epidermis by exocytosis. The infiltrates are mainly composed of T helper cells, cytotoxic CD8+ cells and also neutrophils. The neutrophils in the infiltrates form the spongiform pustule of Kogoj, and Munro microabscess, which is characteristic microscopic finding of psoriasis (Kaneko F et al)[34].

The vascular changes include increase in the number and density of the superficial vascular plexus (Shilpa Gupta et al)[35]. The tortuosity of vessels is increased and venulization of capillary network appears (Rubin's Pathology)[36]. The capillary at the site of

lesions appears like vein and the capillary network is called as High Endothelial Venules (Hani A. et al)[37].

2.1.5. Grading the severity of psoriasis:

The severity of psoriasis cannot be graded like other chronic diseases by natural history. Psoriasis is graded by measuring the clinical remission, number of hospital admissions, consultations, number of flare ups, assessing the skin area involvement at the present time. This grading usually will not give information about social or psychological consequences. Clinically Psoriasis Area and Severity Index (PASI) is widely used and accepted. PASI grading is based on the severity of lesions, along with the specific site and area of involvement. The score ranges from 0-72, where '0' score indicate no disease and '72' score indicates maximal disease (Langley RG et al)[38].

Calculations of PASI score:

Whole body is divided into four sections

- Head (H) - constitutes 10% of person's skin.
- Upper Limb or Arms (A) – constitutes 20% of person's skin.
- Trunk (T) – constitutes 30% of skin.
- Lower Limb or Legs (L) – constitutes 40% 0f skin.

The percentage of skin involved in each section is estimated separately, then it is graded according to the following grade.

- Grade 0 - 0% or no skin is involved.
- Grade1 - 10% of skin is involved.
- Grade 2 - 10 – 20% of skin is involved.
- Grade 3 - 30 – 49% of skin is involved.
- Grade 4 - 50 – 69 % of skin is involved.
- Grade 5 - 70 – 89% of skin is involved.
- Grade 6 - 90 – 100% off skin is involved.

Again in each section the severity is calculated by the three important clinical signs

1. Erythema or redness
2. Induration or thickness
3. Desquamation or scaling

Again the severity parameters are measured on a scale from 0-4. Scale 0 means none and scale 4 means the maximum.

For each section of skin, all the severity parameters are calculated summed up and multiplied by the area score and by the weight of respective section (Dennis Thompson etal)[39].

SECTION	WEIGHT
Head	0.1
Upper Limb	0.2
Trunk	0.3
Lower Limb	0.4

The disadvantage in PASI scoring was the score is non - reproducible. Many modifications of PASI scoring have been developed recently. The adapted PASI scoring is an important modification in which the psoriatic lesion area section is not converted into area grade, but it is calculated in form of continuous variable. This modification improved the power of clinical trial.

The modification commonly used in PASI scoring is Computer aided psoriasis continuous area and the severity score mentioned as cPcASI (Louden BAet al) [40]. In this cPcASI system patient's affected area was photographed and the area is automatically assessed by the computer instead of manual measurement by the physician. Increased scoring in the PASI indicates severe form of disease and the clinical severity is difficult to say by any specific PASI score. To overcome this problem psoriasis severity evaluation system includes Lattice- system physician's Global Assessment (LS- PGA), and the Psoriasis Global Assessment (PGA) system**)** Robinson A et al[41].

2.1.6 Other manifestations:

Psoriatic skin disease is most commonly associated with nail changes. Finger nails are most commonly involved than the toes.

Psoriatic arthritis is the second common clinical manifestation of psoriasis. Apart from psoriatic arthritis , it is associated with a number of co- morbidities like cardio vascular disease, Inflammatory bowel Disease (IBD), obesity, metabolic syndrome, sleep apnoea syndrome, psychiatric disorders like depression, solid tumor of specific sites like lung, kidney, colon, certain lymphomas.

Nail manifestation:

The most common characteristic manifestation of psoriatic nail changes is described below;

- Pitting of nail
- Onycholysis
- Oil spots
- Sub ungula hyperkeratosis

Mild nail manifestation in form of pitting is most commonly seen in psoriatic patients. Severe form of nail changes is usually associated with psoriatic arthritis and psoriatic scalp involvement.

Geographic tongue:

It is otherwise called as benign migratory glossitis. It a local disorder of tongue due to inflammatory of mucous membrane and loss of filliform papillae. It appears as red patches with serpiginous

borders similar to a map. The lesions are asymptomatic and migratory in nature. It is considered as an oral variant of psoriasis since the histological features like acanthosis, clubbing of rete ridges, neutrophil infiltrates and focal parakeratosis are similar to psoriasis (Robyn S Fallen et al) [42].

Obesity and metabolic syndrome:

The important source of IL 6 is adipose tissues. Inflammation in any site of body is associated by certain cytokines derived from adipose tissue and liver and the cytokines are IL - 1, IL – 6, TNF – α[43]. Certain cytokines are common in the pathology of psoriasis and obesity, metabolic syndrome. Psoriasis is associated with these disorders, but it is not revealed which comes first. It is suggested that both these disease has a common genetic background (Sterry Wet al)[44] and also obese psoriatic patient show severe form of disease.

Cardio vascular disease:

The process of atherosclerosis involves inflammation and in psoriasis there is immune mediated systemic inflammation (Kremers HM et al)[45]. Several studies have proved psoriatic patients have increased risk of arterial and venous vascular disease and

myocardial infraction. Metabolic syndrome, common is psoriasis is associated with hypertension, obesity, dyslipidemia, insulin resistance, and patients with psoriasis also have elevated C-Reactive Peptide (CRP), homocysteine, prothrombotic state like high fibrinogen, TNF α, and other cytokines which are all proved risk factor for cardiovascular disease. Local and systemic inflammation results in the pathogenesis of coronary heart disease.

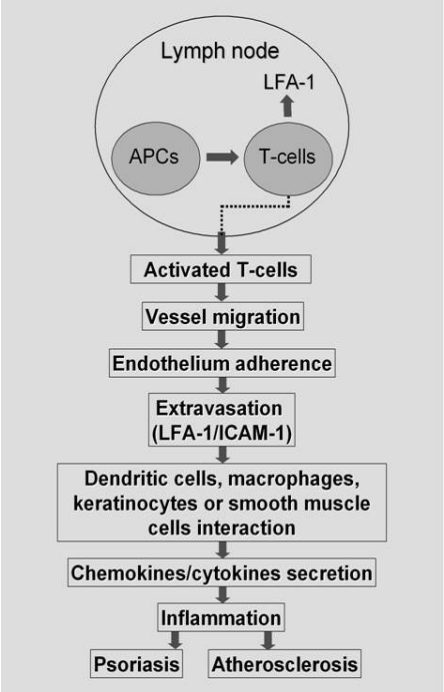
The activated helper T cells release many pro inflammatory cytokines which causes the increased epidermal turn over, can also aids in initiation and progression of atherosclerotic plaques formation (Oliver et al)[46]. The role of cytokines in atherosclerosis is well established.

- TNF –α and IL- 1 causes transient reversible endothelial dysfunction initially. On prolong stimulation it causes permanent irreversible endothelial dysfunction.
- IL- 6 and soluble IL- 2 causes impaired microvascular dysfunction.
- Impaired TNF –α raises the adhesion molecule expression in endothelium, aids in attraction and adhesion of leucocytes. It also stimulates Nitric Oxide Synthase (NOS), and increases the levels of Nitric Oxide (Trac YZhu et al)[47].
- Nitric Oxide increases the oxidative stress in the endothelial cells.

Thus these cytokines causes oxidative stress and inflammation which results in increased apoptosis, formation of atherosclerotic plaque and thrombus formation ultimately resulting in coronary heart disease and stroke. There are several evidence that the pathogenesis of psoriasis and atherosclerosis have a pivotal link.

Both the disease share some common pathologic features. The immunological activities and pro inflammatory cytokines from activated cells play important role in both the disease. Both disease share T helper cell mediated immune compromise and same pattern of T cell activation and expression.

Figure 2.1 Common pathogenesis of psoriasis and atherosclerosis



2.2 Psoriatic Arthritis (PsA):

Psoriatic arthritis is an immune mediated inflammatory musculo skeletal disease showing negative rheumatoid factor in serum. PsA involves the musculoskeletal structures of peripheral and axial joints and also involves entheses, tendon sheaths. The exact proportion of psoriatic skin patients developing arthritis cannot be predicted. On an average the proportion of psoriatic skin patients developing arthritis ranges from 6% to 42% worldwide. In India the proportion of arthritis developed in skin patients ranges from 7-20 percent.

PsA develops in patients with severe skin disease usually, but in some 12- 15% of patients arthritis appears first then in due course of time they develop skin manifestation.70-82% of PsA patients have an average duration of 12 years of skin manifestations, later they develop arthritis. In the remaining of patients both the skin lesions and the arthritis occurs concurrently at the first presentation.

PsA also has no sexual predilection like RA where women are more predilected. It occurs equally in both men and women in the ratio of 1:1. PsA can occur at any age, even children show PsA manifestations but it usually occurs in middle age. Children with

PsA shows increase incidence of developing uveitis and chance of progressing to severe and disabling form of the disease.

2.2.1Clinical presentation:

The involved joints show pain or tenderness, swelling, stiffness and also tenderness around the joints involving the ligaments and tendons. These are the common symptoms and can vary from mild to severe forms. The characteristic of psoriatic arthritis is it tends to follow a relapsing and remitting course (Shah NM et al)[48]. The clinical presentation is usually preceded by skin lesions but in some patients the skin lesions may be occult or left un-noticed by the patient.

Occult lesions should be searched in extensor surfaces, umbilicus, scalp, body folds and nail changes. Enthesitis of Achilles tendon and plantar fascia is also common clinical presentation. Tenosynovitis of PsA usually affects the flexor tendons rather than the extensors tendons. Sacroilitis and conjunctivitis or uveitis is common in HLA- B27 (Duarrani et al) [49].

2.2.2 Classification:

In 1973 Moll and Wright was the first to describe the psoriatic arthritis and classified psoriasis into five subtypes. There is high

degree of overlap between categories within these subtypes and hence show limitation in clinical practice.

1. Distal interphalangeal arthritis (DIP)
2. Symmetric polyarthritis
3. Asymmetric oligoarthritis
4. Spondylo arthropathy
5. Arthritis mutilans

The pattern of arthritis may change from one form to other in due course of time. Patients initially present as asymmetric oligoarthritis initially, the as more joints are involved in due course of time, and they show symmetrical involvement and the pattern changes to symmetric polyarthritis. Similarly the disease initially shows more peripheral joint involvement and later progress to involve the axial skeleton.

In 1994 Veale et al[50] included SAPHO syndrome as a distinct subgroup. SAPHO syndrome is a variety of inflammatory disorder of bone associated with skin lesions. It includes synovitis, acne involving face and trunk, pustulosis involving the palms and soles, hyperostosis and chronic recurrent osteomyelitis[51].

CASPAR (ClASsification criteria for Psoriatic ARthritis):

S. No	Diagnostic point	Score
1	Presence of well-established psoriatic lesions	2
2	A past History of psoriasis, but at present there is no lesions	1
3	A family history of psoriasis, but no present or past psoriatic lesions	1
4	Presence of dactylitis or previous history of dactylitis recorded by a rheumatologist	1
5	Juxtra articular formation of new bone	1
6	Serum rheumatoid factor negativity	1
7	Typical psoriatic nail dystrophy like onycholysis, pitting, hyperkeratosis.	1

It is used in recognized inflammatory joint and articular disease. Inflammatory joint disease is diagnosed with the history of prolonged morning stiffness or stiffness following prolonged immobility, with or without radiological evidence. At least three points from the following defined features establish the diagnosis of psoriasis.

2.2.3. Genetics of psoriatic arthritis:

Family study among the PsA patients suggested that their first degree relatives are in greater risk of developing the disease. Moll and Wright's family studies indicate the recurrence risk is higher among PsA patient's family. There is a strong genetic association for PsA. PsA involves multiple genes and genetic polymorphisms also influence it. Some genes with regard to PsA has been identified and they belong to HLA alleles, MHC class I, Killer Cell Immunoglobulin Like Receptor (KIR)genes, TNFα, IL 1, IL23Receptor genes.

- HLA B13, HLA B16 and its splits HLA B38and 39 are associated with PsA.
- -Cw6 is associated with psoriatic skin disease not with PsA. The association has distinguished a 100 kb telemetric region on a HLA C locus. The true locus is exactly not in HLA C, also involves another gene in linkage disequilibrium (Nair RP etal)[52].
- HLA B 27 and HLA 7 are associated with PsA specifically.
- HLA-C, IL 12 and IL23 R have strong association with PsA.
- Novel PsA a locus has been identified on locus chromosome 4q 27 which anchors IL 2 and IL 21genes.

- HLA B39, HLA B27 in the presence of HLA DR7 and in the absence of HLA DQ 3confers increased risk of disease progression.
- TNF polymorphisms are associated with erosive arthritis and the joint damage progress quickly. It is commonly seen in early onset PsA.
- IL 4 receptor gene is also associated with erosive PsA.

2.2.5 Pathogenesis:

It is an autoimmune disease directed the joints, tendons and ligaments. Chronic auto reactive T cell driven inflammation is the main pathogenesis of PsA. The pathogens encodes the exogenous ligands and these ligands activate the T cell in previous inflammation. This response in the prior episodes of inflammation results in expansion of memory effector T cells. This memory effector T cells recognize stress related self – antigens and initiates and maintains the pathways of inflammation mediated by transcripition factors, activator proteins, production of cytokines and inflammation directed against skin, joints, and musculoskeletal structures.

2.2.5 Link between skin and musculoskeletal inflammation:

Only 13 – 20% of psoriatic skin patients get arthritis and the rest do not develop it. Offidani et al[53] study has proved by MRI and X ray finding has proved that there is a subclinical joint inflammation in many psoriatic skin diseases that may show musculoskeletal syndrome in due course of disease. The activated lesions in the skin enters the sub synovial vessels in the joints promotes inflammation. Some local factors like trauma, stress, infection could promote and initiate innate immunity response that rapidly progress to acquired immunity in setting a T cell expansion in skin, lymph nodes, soft tissues, joints, tendons.

Skin and joint contain common micro anatomic features like differential degree of mechanical stressing, vascular as well as non – vascular tissues and share same antigens. It is very hard to detect the specific genes for skin and joint manifestations because patients with skin disease may or may not develop joint manifestations. Likewise most of PsA patients have cutaneous manifestations. The musculoskeletal and skin is not linked by circulating cells, but by an abberent response to injury in skin and the joint.

2.27. Predictions of worse prognosis:

The course, the severity and response to treatment in PsA is unpredictable. It varies among person to person and even in the same person the sub types vary during the course of disease.

Some of the symptoms and presentation may predict the outcome of the disease[54] and they are

1. Polyarticular involvement
2. Erosive arthritis at presentation
3. Extensive skin lesions
4. A strong positive family history
5. Younger age of onset

2.2.8 Other musculoskeletal features:

- **Enthesitis** – Inflammatory lesions at the site of insertion of tendon into the bone. It is seen in 20- 40% of PsA patients. It is the first sign of clinical presentation of PsA in 4% of patients. Common sites of enthesitis are Achilles tendon, insertion site of plantar fascia at the calcaneous bone, ligament insertion sites in pelvic bones.
- **Dactylitis** – 30-40% 0F PsA patients in due course of time develop dactylitis. Dactylitis is complete swelling of the finger. One or two fingers are involved at a time and it

involves the toes rather than the fingers. Radiographic evidence shows erosive arthritis of the involved finger. The patients later develop flexor tenosynovitis, soft tissue edema, bone edema and also synovitis.

- **Peripheral edema-** Distal extremities one or more show peripheral edema. It usually occurs in asymmetric arthritis prefentially involving lower limbs.It can occur at any time and is due to extensor tenosynovitis and this local enthesitis. There is edema along the whole course of involved tendon. This condition is pain less, but the arthritis is painful and commonly associated with erosive arthritis (C. Palazzi et al) [54]. This condition responds well to oral corticosteroids.
- **Psoriatic onychopachydermaperiositis –** This condition is seen in severe PsA. It involves one or more distal phalanx both in upper limb and lower limb. The involvement of DIP is variable and hard to detect due inflammation and edema of distal phalanx. Common site of this condition is great toe and radiological images of great toe in this condition is called as 'Ivory phalanx'

2.2.9. Pathologic cartilage resorption

Loss of cartilage is common in PsA and its radiological evidence is narrowing of joint space. Levels of Matrix Metalo

Proteinases (MMPs) and their tissue inhibitors Tissue Inhibitor Metalo Proteinases (TIMPs) are elevated in the synovial lining cells and the subsynovial cells in affected joints of PsA patients (C Ribbens et al) [55].

Immune histochemical studies reveals that MMP – 9 in vasculature and MMP – 1, MMP 2 and MMP 3 (Senger DR et al) [56], TIMP 1, TIMP2, m RNA were elevated similar to that of Rheumatoid arthritis. These immuno pathogenic mechanisms that dictate cartilage destruction in PsA are also shared with other Spondyloarthropathies.

Altered bone remodelling:

Bone involvement in PsA is intriguing and has confounded attempts to explain it because it is so heterogeneous event in PsA patients. Altered bone remodelling (Gospodarowicz D et al) [57] and new bone formation includes joint ankylosis, syndesmophyte formation at the sites of soft tissue including the enthesis is common in PsA.

PsA involved sites of bone show diffuse bone marrow edema and histologically evident as osteitis. The diffuse bone edema at the tendon insertion sites is less in comparison to other Spondyloarthropathy.

In DIP bone edema is common and prominent, and in metacarpo phalangeal joints less number is involved and also it is less prominent. In knee diffuse osteolytis occurs typically adjacent to insertion of tendons and it is seen in one third of PsA patient. The osteitis progress to joint destruction. Osteitis related to several TNF transgenic models are conducted. In TNF transgenic models the earliest lesion is seen in stromal compartment of enthesis.

Radiological evidence of affected bone shows large eccentric bone erosions, pencil cup deformities, bone resorption, new bone formation, perostiits, ankylosis peripheral joints. Axial joint shows ankylosis in the peripheral joints and enthesophytes in spine.

4.1. Autonomic Nervous System (ANS):

Autonomic Nervous system is a division of Central Nervous System (CNS) which controls the visceral motor control and ultimately the visceral homeostasis. Apart from skeletal muscle all other organs are innervated by ANS. Survival of an individual is even possible with the absence of ANS regulation but the body's internal homeostasis is severely compromised. Visceral homeostasis is essential for maintaining a dynamic internal environment. Dynamic internal environment is essential for proper functioning of all cells, tissues, and organ systems.

ANS is classically divided into two main divisions

i. The sympathetic nervous system- Thoracolumbar division

ii. The parasympathetic nervous system- Cranioscaral division

Some considers the enteric nervous of Gastro Intestinal Tract (GIT) as a part of ANS, but it is now commonly accepted as a separate entity. ANS basically consists of two sets of neurons. They are

i. The pre- ganglionic neurons also called as connector

ii. The post ganglionic neurons also called as effector

iii. The peripheral ganglion

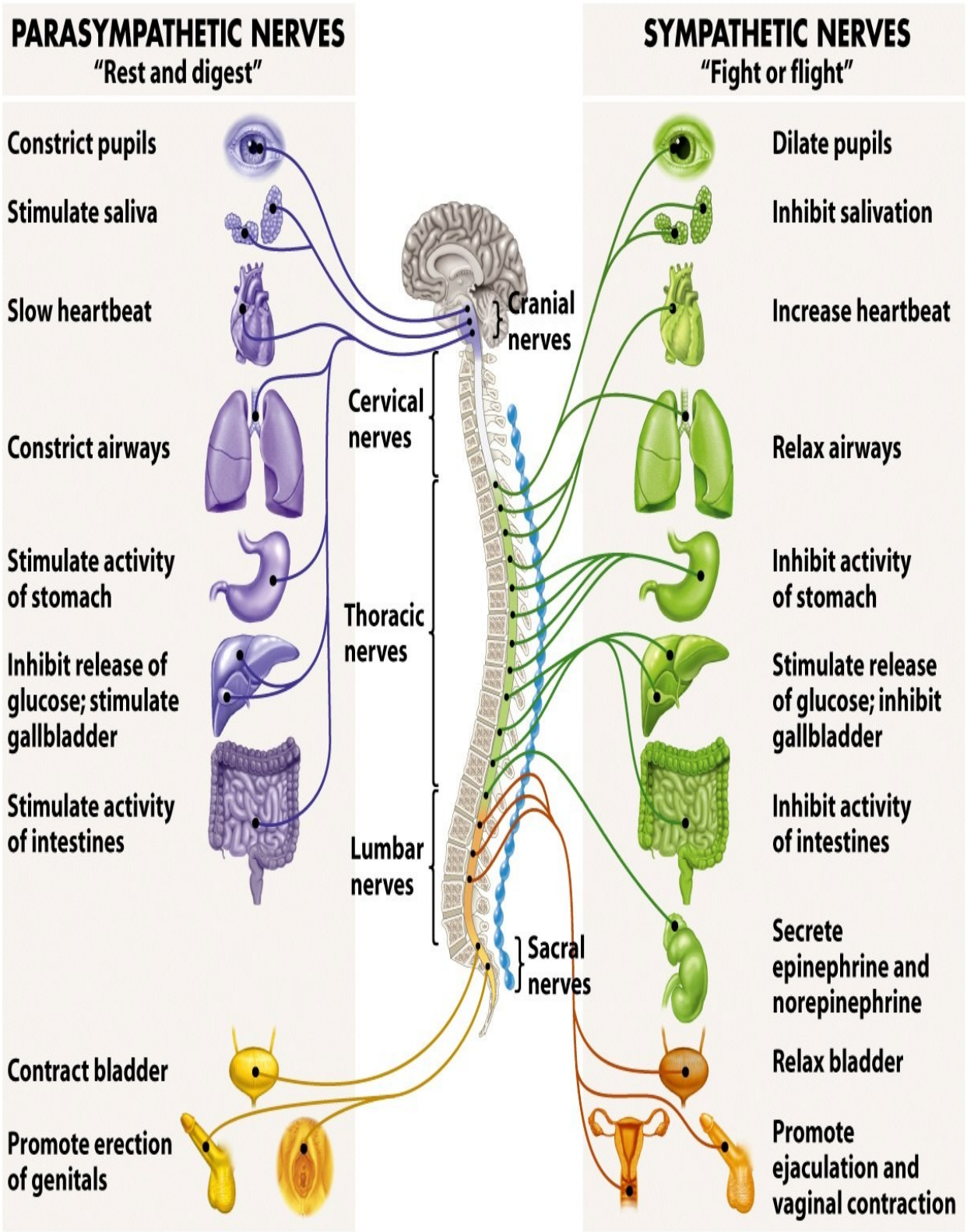


Figure 2.2 Autonomic Nervous System

Autonomic out flow pattern:

The pre- ganglionic neurons are present in brain stem and in the intermediolateral gray horns of thoracic and lumbar segments of spinal cord. The post ganglionic fibers are present in pre and paravertebral chains. The peripheral ganglion is present in the peripheral organs which is characteristic of ANS and these ganglion acts as relay stations. It provides control loops close to the visceral organs innervated by the ANS.

The ANS innervation is different from somatic innervation, ANS involves both the neurons pre – ganglionic and post-ganglionic nerve fibres and they link the central portion and the visceral structure. ANS is noted for the relay that it makes between the CNS and the visceral organ they serve.

Functional and pharmacological features of ANS:

Parasympathetic and sympathetic divisions of ANS differ anatomically, morphologically, physiologically and pharmacologically. Though they operate reciprocally and antagonistically their actions are highly controlled and integrated thoroughly.

Sympathetic activation causes the individuals to mobilize the individual and spend the energy and associated with ‘ergo tropic ’

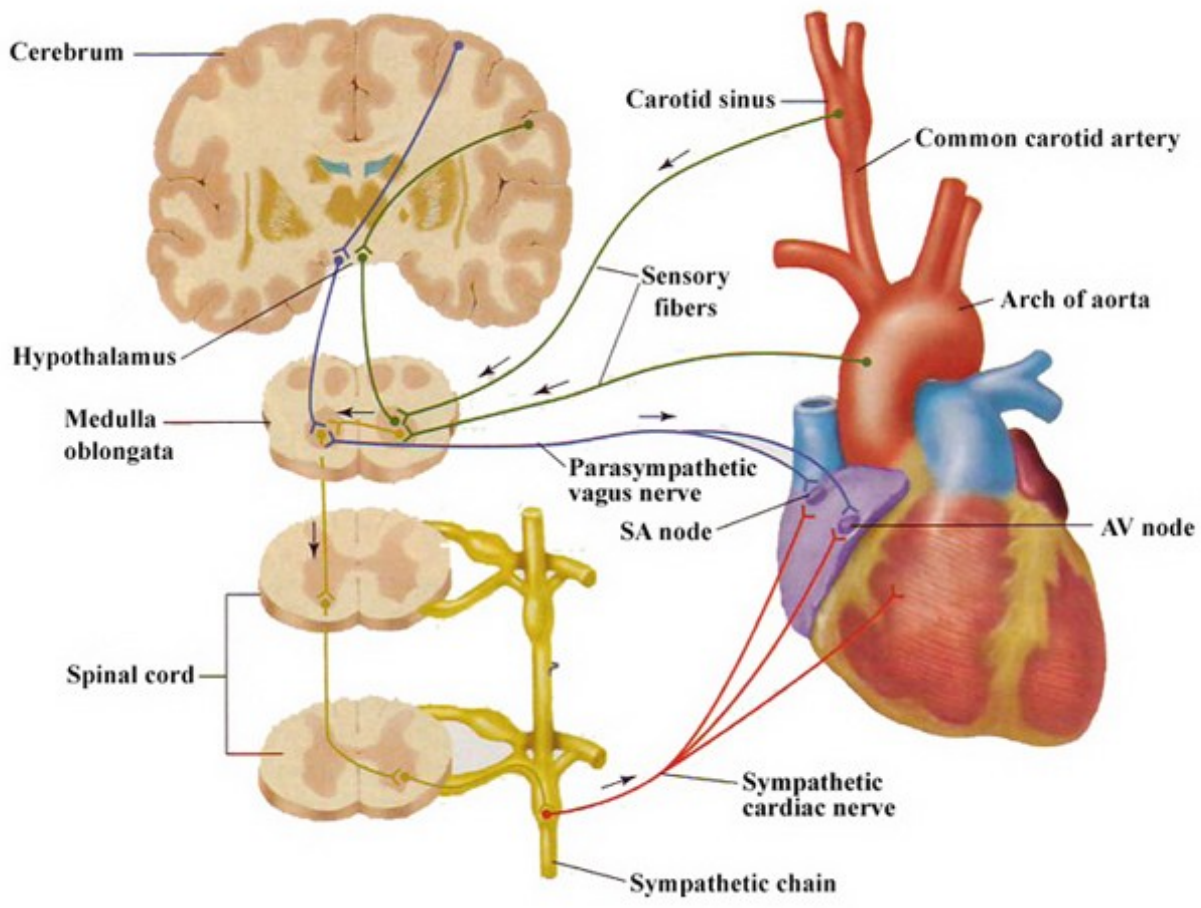
(ergo means energy and tropic means releasing) . In contrast parasympathetic activation causes conservation of energy and the operation of vegetative functions to conserve energy. Thus function provided by parasympathetic system activation is concerned with day to day vegetative actions and hence parasympathetic activation is called ‘tropotropic (trophos means nourishment).

The main neurotransmitters released from ANS nerve fibers are Acetylcholine and Nor-Adrenaline. All the preganglionic fibers both sympathetic and parasympathetic releases Acetylcholine. All post ganglionic parasympathetic nerve fibers and cholinergic postganglionic nerve fibers secrete Acetylcholine. Adrenergic post ganglionic fibers secrete Nor-Adrenaline.

Autonomic control of Heart:

Heart is innervated by both sympathetic and parasympathetic nerves. The sympathetic preganglionic fibers originate in the intermediate horns of upper 4 or 5 thoracic spinal segments, relay in stellate ganglion, reach heart and innervate pacemaker, conduction system and also atrial and ventricular muscles. The vagus (parasympathetic) nerve originates in the dorsal nucleus of vagus in medulla, ends in ganglia close to SA and AV node and the short post ganglionic nerves supply the pace maker, the conduction fibers, and also innervates the muscle fibers but sparsely.

Figure 2.3 ANS innervation of heart



The vagus is cardioinhibitory and on stimulation releases Acetyl choline from the post ganglionic nerves which makes the cardiac fibers less excitable. The acetylcholine increases the potassium permeability and cause increased potassium efflux resulting in hyperpolarization of membrane. The end result is the tissue becomes less excitable. On vagal stimulation the rate of pacemaker impulses are reduced, conduction in the conduction tissue is slowed, the force of contraction of atrial muscles is reduced and the ventricle muscle is least affected.

Effect of Vagal stimulation on heart

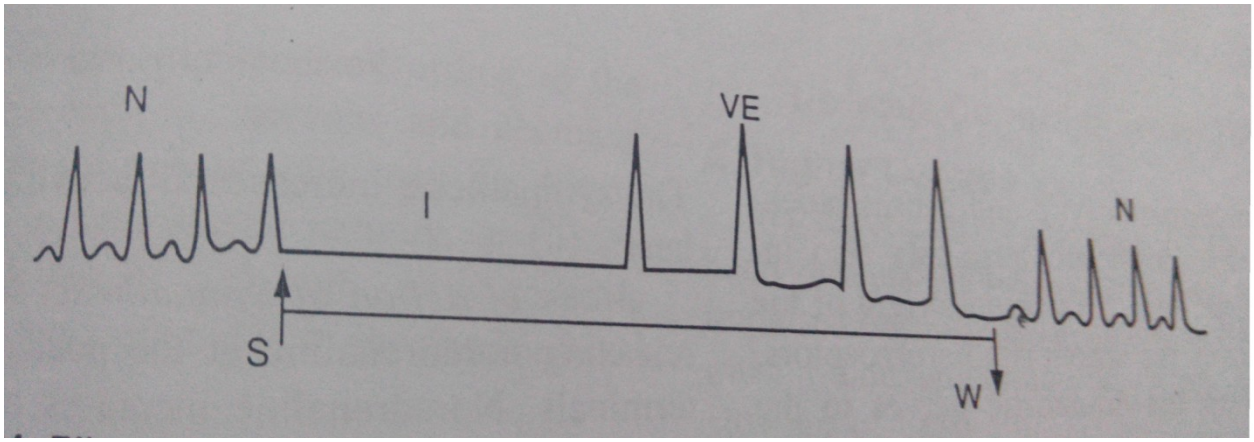


Figure 2.4. N- Normal heart beats, S- Stimulation, I- Inhibition, VE- Vagal escape, SW stimulation to Withdrawal.

The impulses from cardio respiratory region of medulla passes continually to the heart via the vagus and keeps a check of heart rate and that is called as vagal tone. The vagal tone originates from sinoaortic baroreceptors, and the impulses reach medullary cardio inhibitory center and send impulses to heart via vagus. Sino aortic denervation causes abolition of vagal tone.

The sympathetic stimulation causes increase in heart rate (chronotrophic), increase in force of contraction (inotropic) and also increase in conduction velocity (dromotropic). It also increases the excitability (bathmotrophic) and reduces the relaxation of cardiac muscle (lusiotrophic).It releases nor adrenaline from the post ganglionic nerve terminals. It reduces the potassium efflux and causes the opening of transient calcium channels in SA node. The

opening of L channels in the cardiac muscles increase the force of contraction. These actions are brought by β 1 receptors (Frielle T et al) [58] in the cardiac tissue.

There is some degree of sympathetic tone along with vagal tone and sympathetic denervation reduces the heart rate considerably. These impulses originate from the medulla and from the hypothalamus also. This tone also keep the vessels in partially constricted state and plays role in maintaining the vascular resistance and in maintaining the blood pressure.

Normally there exists a balance between sympathetic and parasympathetic tone and called as sympathovagal balance. Intrinsic heart rate is said to be heart rate of a denerved heart and it is 100-110 beats/ minute. The normal adult resting heart rate is around 72 beats / minute and it is said that under resting conditions the parasympathetic tone predominates.

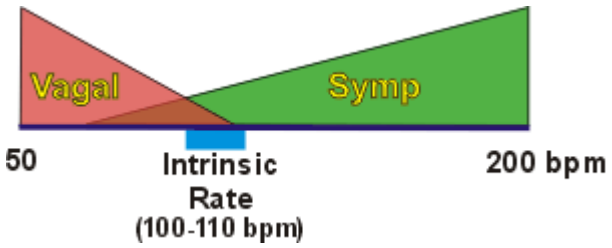


Figure 2.5

Reflex control of heart rate:

1) Sinoaortic baroreceptor reflex

Baroreceptors are mechanoreceptors which are situated in the tunica adventia of carotid sinus and transverse arch of aorta, supplied by coiled myelinated pressure sensing nerve endings of glossopharyngeal and vagus nerve respectively. The afferents reach the Tractus solitaries of medullary cardiovascular center.

At normal blood pressure there is a low frequency of impulse discharge in these nerves and it is responsible for the vagal tone. Any increase in blood pressure (upto 180 mmHg) increases the discharges from the baroreceptors which reduce the heart rate and vice versa occurs in decrease in blood pressure (upto 60 mmHg). Bilateral sinoaortic denervation increases the heart rate and similarly bilateral clamping of carotids below the carotid body increases the heart rate.

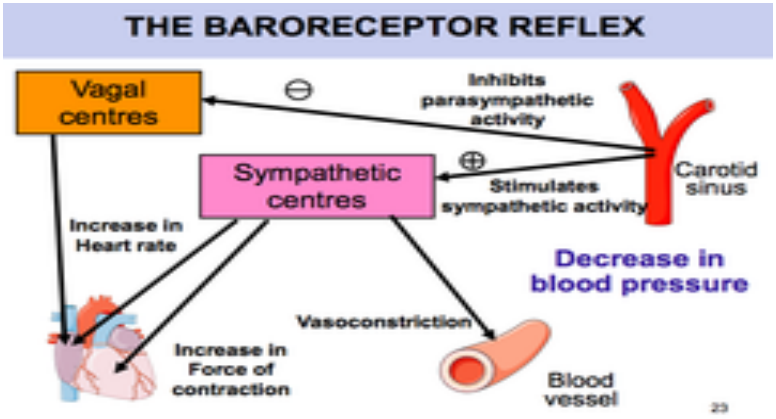


Figure 2.6: Functioning of baroreceptors

2) Reflexes from peripheral chemoreceptors:

Chemoreceptors is stimulated by hypoxia, hypercapnia, and hydrogen ions. On stimulation per se it causes decreased heart rate, but indirectly the stimulants of chemoreceptors increases the heart rate.

3) Bainbridge reflex:

Venous engorgement of the right side heart causes increase in heart rate and brought by stretch receptors and the effect is abolished by bilateral vagotomy. The afferent is vagus and the efferent is both sympathetic and vagal nerves.

4) Sinus Arrhythmia:

Variations of heart rate occurs during the phases of respiration. The heart rate is increased during inspiration and reduced during expiration. It is well appreciated in children and young adults and diminishes with age. The reason of increased heart rate during inspiration is

- Alteration in venous return and blood pressure during various phases of respiration.
- Irradiation of impulses from respiratory to cardio inhibitory centers.
- Reflexes from stretch receptors in lungs which is changed during the phases of respiration.

5) Peripheral receptor stimulation:

A strong somatic receptor stimulation may alter the heart rate. A painful stimuli or a strong thermal stimulation increases the heart rate.

Figure

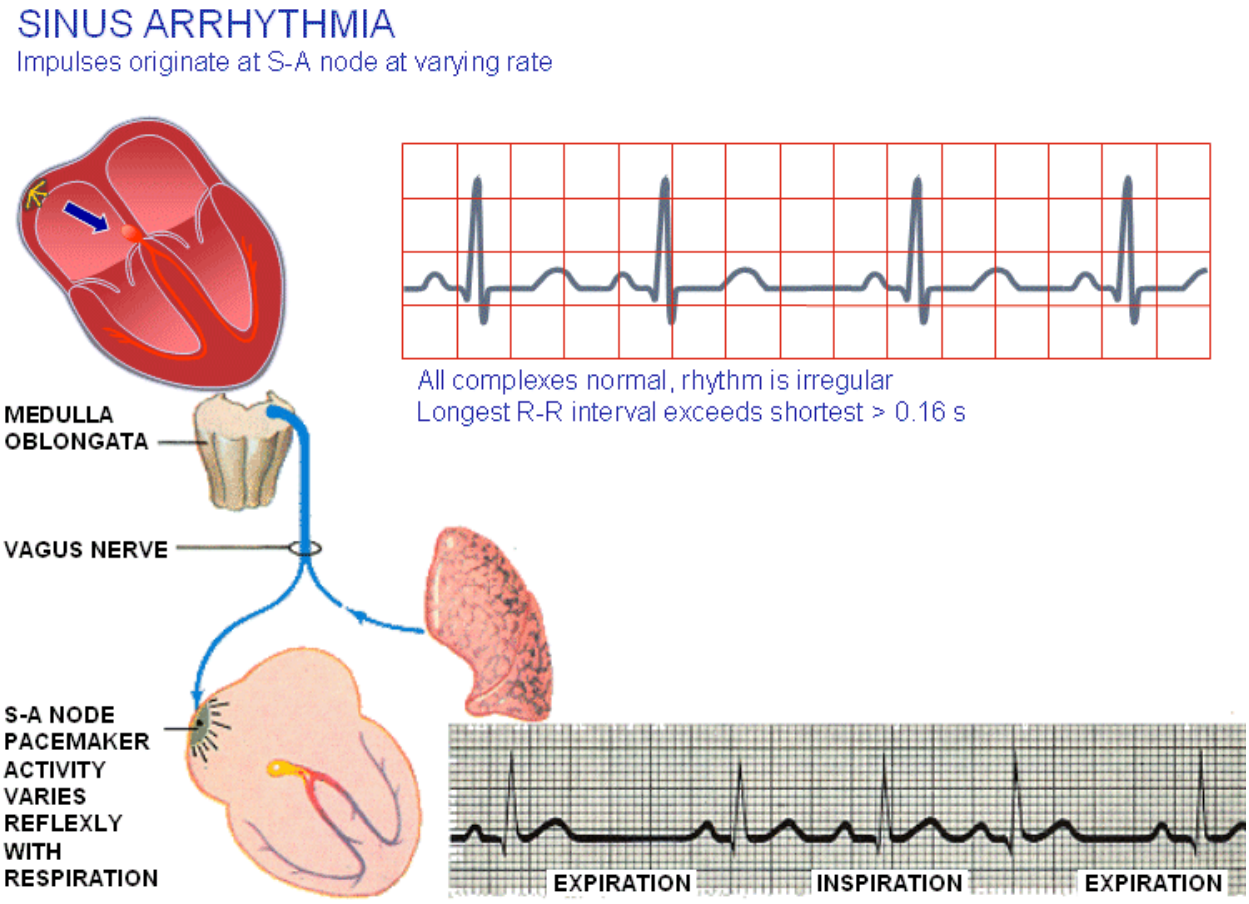


Figure 5.7 sinus arrhythmia

5) Bezold –Jarish reflex- marked stretching of left ventricle causes reduction of heart rate.

6) Oculocardio reflex: pressure in eyes increases the vagal tone and reduces the heart rate.

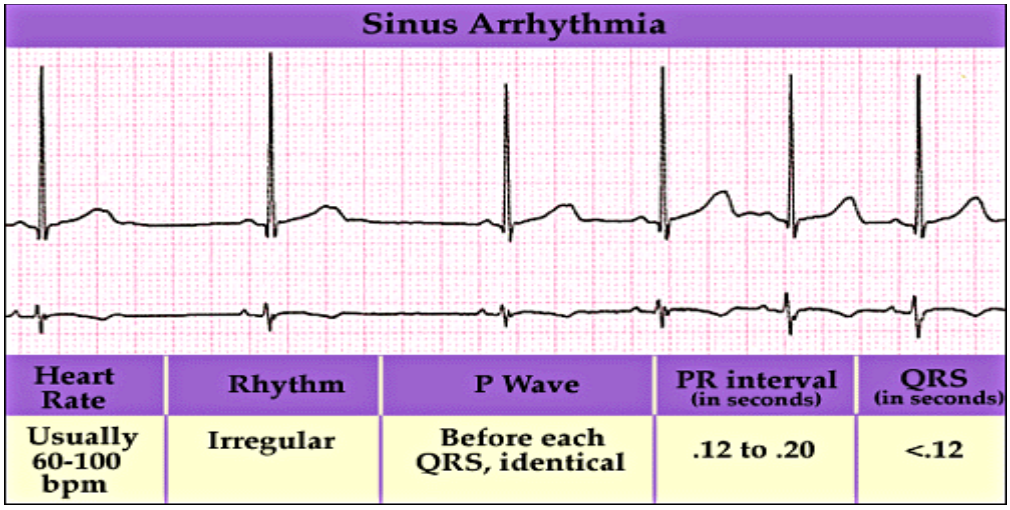
The other factors regulating the heart rate is hormones (catecholamines, thyroxine, vasopressin), temperature, levels of oxygen, carbondioxide, hydrogen ions.

Heart Rate Variability:

The variation of heart rate interval between two beats and between the instantaneous heart rate is known as 'Heart Rate Variability' (HRV). The reason for normal fluctuation of heart rate is respiratory arrhythmia, baroreceptor reflex, thermoregulation and circadian rhythm.

This HRV is a normal physiological phenomena and a healthy heart shows high variations in HRV. Thus depending on the sympathovagal tone, the heart shows beat to beat variation and its measure in form of R- R interval is a measure of autonomic function. A reduced heart rate variation indicates defective balance between sympathetic and parasympathetic nervous system, and the ability to maintain homeostasis is impaired (Martin G. et al) [59]. A reduced HRV suggests a vulnerable state in which the stress which may be internal or external cannot be handled.

Figure 2.8 Normal HRV due to Sinus Arrhythmia



The changes in ANS is reflected in HRV, even though the physiological parameters lie within normal standard range. It identifies distress before appreciable clinical findings and reflects the early loss of internal homeostasis.

Though many factors fluctuate heart rate resting HRV measures the fluctuation of heart beat during respiration (respiratory arrhythmia). The phenomena of normal variation diminishes with age and also in diseased conditions.

HRV is considered as a marker of parasympathetic system and it is proved by using parasympathetic markers (Task forces)[60]. It is a non- invasive tests which helps in evaluation of the ANS and its integral functions.

HRV measurement:

The simplest method of evaluation of HRV is time domain measures. At a given point of time the heart rate and the interval within the successive beats is determined by time domain values.

In a continuous ECG record, each of the QRS complex is identified and all the intervals between consecutive QRS complex is determined. That interval is called as Normal- Normal (NN) intervals, by which the instantaneous heart rate is determined. The commonly used simple time domain factors are mean NN interval, mean heart rate and Standard Deviation of NN (SDNN).

SDNN is the square root of variance and it represents the total power of the spectral analysis. It reflects all the components which occur cyclically during the variability period of recording. Better results of SDNN is brought if the period of monitoring is long, like 24 hour monitoring. In short duration of recording the variance of total HRV increases[60].

Power Spectrum Density Analysis:

The information of variance (power) distribution is provided by power spectral density analysis. The analysis is carried by non-parametric methods. The advantage of non-parametric method is its simple algorithm in form of Fast Fourier Transform (FFT) and a high processing speed.

The important spectral components in short term recordings around 2- 5 minutes is

1. Very Low Frequency(VLF) – 0.00- 0.04Hz
2. Low Frequency(VF) – 0.04- 0.15Hz
3. High Frequency(HF) – 0.15- 0.4 Hz

The measurements of VLF, LF and HF components can be in absolute power or in normalized units (n.u). HF and LF is measured in n.u highlights the balanced and controlled behavior of the two branches of ANS.

Role of ANS in psoriatic skin and PsA:

The association of nervous system and immunity is well established. The neural and the immunal cells communicate with each other by cytokines secreted by activated immune cells and by neurotransmitter of the neural fibers to regulate the level and magnitude of immune response. Thus to maintain immune homeostasis proper neural control is essential.

The important mechanism responsible for this synchronization involves the ANS is nor -epinephrine , which acts as a messenger from the mind to all organ systems including immune system. The cytokines acts on receptors in vagus or in the sympathetic nerve terminals or in the CNS(Ionescu G et al) [61] . The CNS communicate

the immune cells by activating sympathetic nervous system or by the Hypothalamo – Pituitary- Adrenal axis and the neuro transmitter is nor adrenaline or a corticosteroid respectively.(Zangeneh FZ et al)[62]

The lymphocytes express receptors which binds nor adrenaline and corticosteroid, so that these ligands activate intracellular signalling pathway and regulates the immune cells. Thus a there exists bidirectional communication, in which one controls over the other (Nance DM et al) [63].

In psoriasis due the activation of immune cells there is excessive release of cytokines which in turn causes excess corticosteroid and nor adrenaline secretion to control the inflammation. Since the main cause of trigger is unknown and not eliminated, (which is genetically determined) the levels of sympathetic hormones are elevated. It is established that the levels of hormone is high and the receptors in immune cells are down regulated. When psoriatic patients receive β- blockers there occurs an exacerbation of lesions in skin and in musculoskeletal manifestation. The reason is the excess hormones tries to put a check over the activated immune cells and when the inhibition is gone there occurs an exacerbation (Halevy S et al) [64].

2.4 Vascular Endothelial Growth Factor (VEGF):

New vessel formation or neovascularization includes two separate components, vasculogenesis and angiogenesis. Classical vasculogenesis occurs during embryogenesis. The new blood vessels are formed from hemangioblasts of primitive mesoblastic cells. During vasculogenesis A primitive network of endothelial tubes are formed which are remodeled into circulating system. The endothelial tubes undergo proliferation, migration, branching and regression.

Angiogenesis is a physiological process of formation of new blood vessels. It is different from vasculogenesis. In angiogenesis the blood vessel arise from pre-existing vessels. Angiogenesis may occur by capillary sprouting or non - sprouting of new blood vessel. Mature endothelial cells divide and incorporate into pre-existing capillaries. Angiogenesis a physiological process vital for growth and development. It also plays vital role in wound healing and repair. The process of angiogenesis may also occur in pathological condition like diabetics, hypertension, inflammatory disease and malignancy and also plays an important role inflammation.

Asahara et al[65] described the existence of endothelial progenitor cells in adult human blood. Normally these progenitor cells reside inside the bone marrow. These progenitor cells are

mobilized into circulation by the signaling cytokines or angiogenic growth factor signal molecules.

Bone marrow derived endothelial progenitor cells recruit to the sites of angiogenesis, where they undergo multiplication and differentiation into mature cells, which combines and forms new blood vessel. But some studies says even the mature adult endothelial cells undergo mitotic division and they undergo increased and rapid divisions at the time of angiogenesis. The estimated surface area of human adult endothelial surface area is 1000 m^2. On an average of 10,000 endothelial cells one will undergo cell division at a given time normally. During angiogenesis there is accelerated range of mitosis at that site.

Physiological angiogenesis occurs in

- Growth and developmental period
- Wound healing and repair
- Repair of corpus luteum
- Formation of placenta

Some examples of pathological angiogenesis

- Tumour formation –benign and malignant
- Senile macular degeneration of eye
- Endometriosis

- Tumour metastasis
- Inflammatory conditions like psoriasis, rheumatoid arthritis
- Diabetes
- Hypertension

Angiogenesis is controlled by both activators and inhibitors. Among the activators there exits three family of activators and their receptors are also included.

1. VEGF/ VEGF Receptor – the most studied family of angiogenesis activator
2. Angiopoietin / Tie system – important in vessel maturation and quiescence of endothelial cells.
3. eph/ Ephrin system- positional guidance cues and arterio venous asymmetry.

Acidic and basic Fibroblast Growth Factor and many others also aids in angiogenesis but are included in the family of angiogenesis inducers.

Vascular Endothelial Growth Factor (VEGF) family includes five members and they play important role in angiogenesis, vasculogenesis and also lymphogenesis.

The members of VEGF family are

1. VEGF A or simply VEGF
2. VEGF B

3. VEGF C

4. VEGF D

5. Placental Growth Factor (PlGF)

All these are proteins that contains signal sequence that is cleaved during biosynthesis. Splicing of their mRNA creates multiple isoforms. Multiple isoforms exists especially to VEGF, VEGF B, PlGF. There are three receptors which are protein kinases dependent and they are VEGF R1, VEGF R2, and VEGF R3. Two non- enzymatic receptors is also present which is Neurophilin 1 and Neuropilin 2. The Neurophilins are receptors involved in neuronal development. The form receptors for semaphorins, which are molecules involved in axonal guidance (Kolodkin AL et al) [66].Many cytokines induce the expression of VEGF in cells and they are Platelet- Derived Growth Factor, fibroblast growth factor and Transforming Growth Factor(Ferrara N et al)[67].

Discovery of VEGF

Senger et al[68] in 1983 isolated VEGF from ascites protein and found to induce angiogenesis in cell culture. This factor which was named as VEGF later was found to cause increased permeability. Ferrara and Henzel[67] purified this protein from bovine cells and found to enchase vascular endothelial mitogenic property

(Gospodarowicz Det al) [69]. Connolly et al[70] isolated and purified this factor from guinea pig carcinoma of liver and proved its vascular permeability enhancing activity. VEGF is specific in enhancing mitosis of endothelial cells and fails to cause mitosis of smooth muscle cells, fibroblasts, lymphocytes, chondrocytes and melanocytes. Molecular weight of VEGF was estimated by denaturing gel electrophoresis. The molecular weight under non-reducing conditions is 46kDa and under reducing conditions was 23kDa. The molecular weight shows slight variation because of N-glycosylation, partial proteolysis and isoforms due to alternate pre-mRNA splicing. It was thought as permeability factor as it increase the permeability of vascular endothelium by forming intercellular gaps, vacuoles, vesico- vascular organelles and fenestrations.

Functions of VEGF

- The first and important function of VEGF is angiogenesis. It aids in angiogenesis by

1. Increased migration of endothelial cells
2. Increase in mitosis of endothelial cells
3. Increase in Matrix Metallo Proteinase activity. It induces the expression of proteases implicated in pericullar matrix degradation during neo vascularization (Pepper MS et al)[71].

4. Increase in αvβ3 activity. It is an integrin acts a receptor for phagocytosis and the phagocytic cells are macrophages and dentritic cells.
5. Creation of lumen in the new blood vessel, by resorption and regression.
6. It is a survival factor for endothelial cells.
7. It increases the permeability of endothelial cells to plasma and plasma proteins. This action is essential in neovascularization and it happens without any damage to endothelial cells, with preserved integrity.

- Another important function of VEGF is chemotaxic to macrophages and granulocytes.
- VEGF also causes vasodilation by induction of endothelial Nitric Oxide Synthase (e-NOS) and causes release of nitric oxide from the endothelial cells.
- VEGF provokes Hematopioetic Stem Cell mobilization from bone marrow.
- It induces osteoblast mediated new bone formation (Ferrara et al)[72].
- VEGF also plays role in neuronal protection (Storkebaum et al) [73].

Structure of VEGF:

VEGF is a protein molecule and the size varies and major protein size is 21 kDa. The chromosomal location of VEGF is on short arm of 6^{th} chromosome and the locus is 6 p 23(Wei MH et al)[74]. The splice variants for VEGF are 121, 145, 165, 183, 189 and 206. Of these splice variants 165, 183, 189 and 165 binds to Heparan Sulphate Proteoglycans. The major mRNA transcript sizes are 3.7 and 4.5.

The expression of VEGF is seen first in anterior portion of embryo of mouse. VEGF directs the migration of cells showing receptors for VEGF- R1 and VEGF- R2. The largest human precursor VEGF protein contains 232 aminoacids. A signal sequence contains 26 residues which on removal yields a mature protein containing 206 aminoacids. There are at least six isoforms of VEGF, showing variable amino acid number which are produced due to alternate pre mRNA splicing.

1)*VEGF121- shows relatively free diffusing among tissues

2) VEGF 145

3)*VEGF 165 –partially bound to Heparan Sulfate ProteoGlycans (HSPGs)

4) VEGF 183

5)VEGF 183

6)*VEGF 189 completely sequestered by HSPGs making them a reservoir of VEGF which can be mobilized by proteolysis in the ECF.

*These are the predominant form secreted by most cells.

Muller et al[75] studied the structure of human VEGF using X-ray crystallographic structure. It contains antiparallel homodimer covalently by two sulfide brides which bridges between cysteine 51 and cysteine 60. The predominant feature of each monomer is the cysteine knot motif. This cysteine knot motif consists of a ring of eight residues formed by di- sulfide bridges.

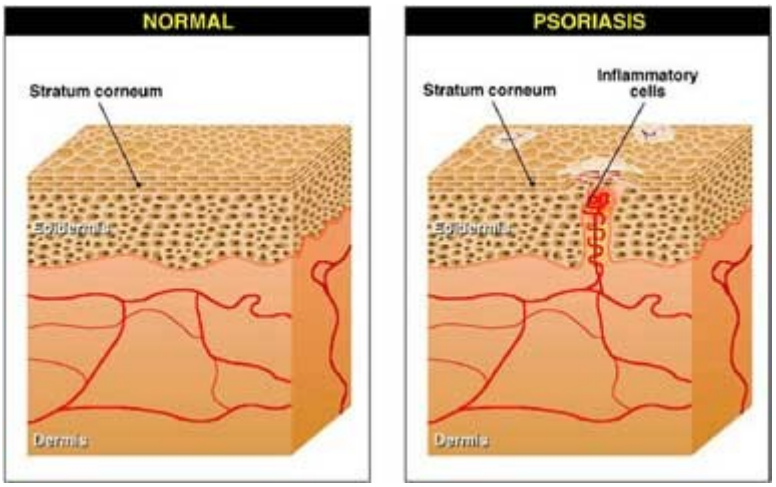
Hypoxia and VEGF:

Hypoxia a potent inducer of VEGF and it induces via Hypoxia Inducible Factor (HIF) which is a regulatory element of VEGF gene. Degradation of HIF is inhibited in hypoxia and HIF prolyl hydroxylases are stabilized and hetreodimerizationof HIF occurs. It is called as Aryl Hydrocarbon Nuclear Translocator (ARNT). All these complex components bind to Hypoxia Responsive Element (HREs) and intiate transcription of many genes of which VEGF AND VEGF R1 gene are important and aids in angiogenesis to overcome hypoxia(Yuxiang Liu,etal)[76].

VEGF in Psoriasis

There are many first messengers in angiogenesis, including cytokines, but the role of VEGF is very important in psoriasis. Psoriasis is an immune mediated hyperproliferative disease of epidermis. The hyper proliferative epidermis is supported by increased dermal angiogenesis and there is over expression of VEGF in seen in the dermal vessels. A genetic experiment on mice showing over expression of murine VEGF is conducted. The mice showed typical features of psoriasis. Histologically it shows poor differentiation of epidermis, dermal thickening, increased density and thickening of dermal blood vessels and venulization of capillaries. These are classical psoriatic features.

Figure 2.9 Increased Angiogenesis in Psoriatic skin



VEGF in PsA

Similar to skin findings VEGF is over expressed at the site of inflammatory joints and soft tissues, tendons. VEGF levels in synovium of affected joints is increased and these levels are reflected in the serum. In PsA the levels of serum VEGF also reflects the disease activity. Serum VEGF is also elevated in Rheumatoid arthritis and in SLE.

AIM AND OBJECTIVES

AIM

To study and compare the autonomic nervous system (ANS) functions and serum Vascular Endothelial Growth Factor (VEGF) in psoriatic skin and psoriatic arthritis (PsA) patients.

OBJECTIVES

1) To study the ANS functions of psoriatic skin and PsA patients.
2) To compare the ANS functions between psoriatic skin and PsA patients.
3) To study the level of serum VEGF levels in psoriatic skin and PsA patients.
4) To compare the serum VEGF levels in psoriatic skin and PsA patients.
5) To correlate the serum VEGF levels and ANS function in psoriatic skin and psoriatic arthritis patients.
6) To evaluate the role of ANS function and serum VEGF levels in the pathogenesis psoriatic skin and psoriatic arthritis.
7) To determine the role of ANS and serum VEGF levels in progression of psoriatic skin disease into PsA.

MATERIALS AND METHOD

The study is conducted in the Institute of Physiology and Experimental medicine, Madras Medical College, Chennai, in the Department of Dermatology and in the Department of Rheumatology, Government Rajiv Gandhi Hospital, Chennai. The study is conducted only after the approval of Institutional Ethical Committee.

The study contains three groups - controls, psoriatic skin and psoriatic arthritis patients. All the three groups contain age and sex matched individuals to avoid bias caused by age and sex. The duration of study is five months from May 2014 – September2014.

4.1 Selection of cases:

Fifteen male and fifteen females are randomly selected in each group and the age of the individuals should lie between 20- 40 years. In psoriatic skin disease the diagnosis is based on clinical presentation, and the signs and symptoms. There is no separate criteria for diagnosis of the disease and biopsy is not necessary. In the PsA group the CASPER criteria is used for the diagnosis. The disease shows no sexual predilection and male, female ratio is 1:1

and hence the study group contains equal number of male and female.

Inclusion criteria for psoriatic skin patient

1) Age group between 20- 40 years
2) Clinical evidence of psoriatic lesions, which may be of any severity
3) The lesions may be new or old or healing
4) Newly diagnosed or a chronic patient with or without treatment
5) The disease may be in remission, exacerbation or a chronic form

Exclusion criteria for psoriatic skin patient group

1) Presence of arthritis or evidence of musculoskeletal symptoms in form of myalgia, enthesitis
2) Presence of infection
3) Pregnancy or postpartum period
4) Diabetes, hypertension, malignancy
5) Established coronary heart disease
6) Renal failure
7) Hepatitis
8) Evidence of interstitial lung disease
9) Presence of any other ill effects due to prolonged medications.

Inclusion criteria for PsA group:

1) Age 20- 40 years
2) Seronegative arthritis satisfying CASPER criteria
3) PsA of any subtype according to Moll and Wright classification
4) Presence of skin or nail psoriasis with arthritis
5) Absence of skin disease but fits in CASPER criteria
6) Newly diagnosed, or a chronic disease
7) The disease may be in remission or in acute exacerbation

Exclusion criteria for PsA group:

1) Presence of infections
2) Pregnancy or postpartum period
3) Presence of other chronic illness like hypertension, diabetes, hepatic or renal disease
4) Presence of malignancy
5) Coronary Heart Disease

Thirty individuals in each group satisfying these criteria is included in the corresponding group.

Selection of controls

Fifteen male and female healthy individuals without any acute or chronic disease like infections, hypertension, diabetes, CHD, malignancy, hepatic renal or lung disorders.

Before the study the participants should be informed about the purpose of study and the procedure is explained. An informed consent is obtained from both the study and the control groups. Detailed history is elicited and complete examination including the general and systemic examination is performed. Under aseptic conditions blood samples are collected and stored under -20^0 C. Since the study period is 5-6 months and the blood samples has to stored properly. All the participants should undergo a battery of ANS function tests.

4.2 Study design:

Case control study

4.3 Materials essential for ANS function tests:

- ❖ INCO Niviqure Ambulatory Digital ECG recorder.
- ❖ Sphygmomanometers
- ❖ Hand grip dynamometer
- ❖ 4-6^0C water in a basin

4.3.1 Description of the Instrument

The instrument is INCO NiviqureAmbulatory Digital ECG Recorder, which is a multi-load,solid state standalone digitalized and computerized recording system. It acquires and analyses and also stores the ECG data over a long period.

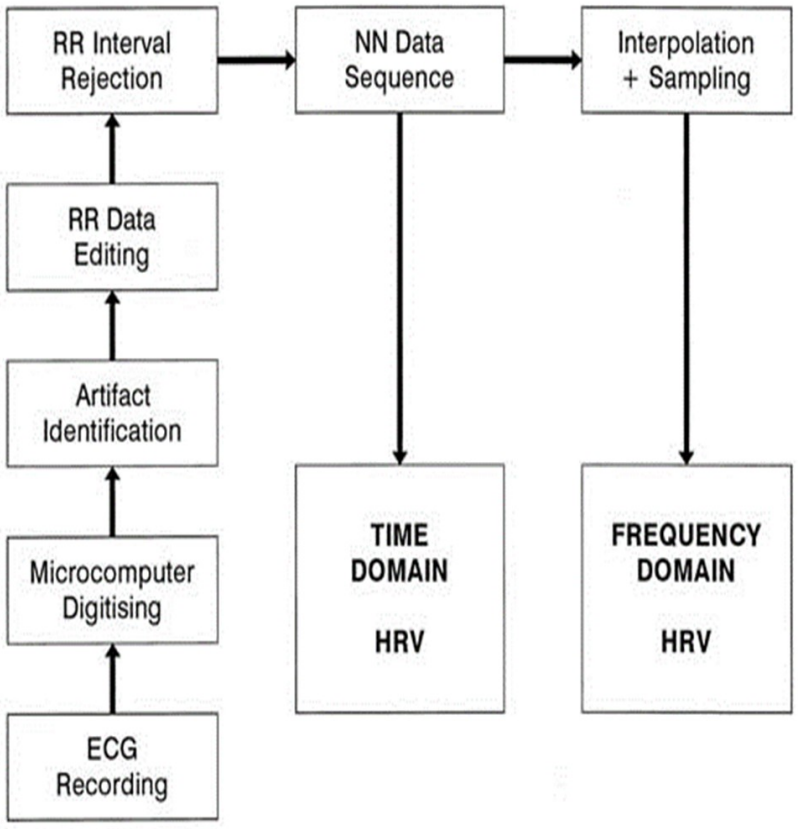
The data acquired is stored in flash memory then it is later can be downloaded and analysed. The data is transferred from the memory module to the computer through a compatible module which is an interface RS233C.

This instrument has powerful processing software for online ECG study, off line data replay, data storage and data study and transfer to other statistical software analysis and also FFT analysis.

Thus the ECG data is initially recorded in the INCO Niviqure Ambulatory Digital ECG Recorder and then transferred to the computer system and the data is processed by eliminating the artefacts and the selected segment is analysed for HRV.

The following flow chart gives the summary of each steps used for recording and processing the ECG data for the analysis of HRV.

Figure 4.1



4.4 Methodology

All the ninety control and study groups are subjected to resting heart rate variability and a series of ANS function tests. The ANS function tests include changes in HRV and blood pressure when subjected to some physiological manoeuvres.

There are many internal and external factors can affect the result of ANS functions. These are the following precautions to be taken while performing the ANS function tests.

- The individuals should be comfortable, relaxed, relived from anxiety without any painful events.
- The ANS function tests should be carried preferably in the morning (8AM- 12 AM) after breakfast.
- Any compressive garments causing discomfort should be avoided.
- Drugs altering ANS should not be used.
- The subjects should avoid coffee, tea, cigarettes, alcohol before the tests
- The bladder should be empty before the test.
- The test should be performed in a quiet, slightly dark room with a controlled and comfortable temperature.
- Mobile phones and other electronic gadgets should be removed and placed at least 5 feet away from the subject.
- The subjects are instructed about the various procedures and allowed to practice the manoeuvers.
- The electrodes should be placed in the following positions

The exploring electrodes should be placed in the left and right subclavicular position in the mid clavicular line. The last exploring electrode should be in the middle of left sub costal region. The reference electrode should be in the right subcostal region.

The period of rest should be around 30 minutes during which the 10 minute resting HRV is recorded continuously. After recording ECG for 10 minutes, continuous 5 minute (320 s) is selected for short term ECG analysis.

The ECG data is screened for artefact and then edited. The data is fed to the software of HRV analysis.The digital converter analogue of resting ECG data is done by using AD converter. The sampling frequency is kept as 1024/ s. the converted ECG data is analysed by power spectrum using Fast Fourier Transformation (FFT). Mean Heart Rate, SDNN, normalized Low Frequency (nLF), normalized High Frequency(nHF), and LF/HF ratio is estimated.

4.5 Deep breathing:

Respiratory arrhythmia is the basic principle behind the tests. To make the respiratory arrhythmia most prominent the respiratory rate should be 6/minute. The individual should breathe at a rate of 5s for inspiration and 5s for expiration. The average acceleration during inspiration and average deceleration during expiration is calculated. The difference between them is calculated as E/I ratio.

The acceleration and deceleration is calculated by the RR interval during inspiration and expiration.

This deep breathing procedure evaluates the parasympathetic nervous system integrity.

Procedure:

The subject should be comfortable and the minimum of 15 minutes of rest should be given between the various ANS tests. The inspiration and expiration should be slow and last for 5s.A minimum of 6 cycles should be performed

E/I Ratio = Longest RR interval during expiration/
Shortest RR interval during inspiration

The results depend on age, sex, body procedure and drugs.

4.6. Valsalva manoeuvre:

This procedure evaluates the functioning of baroreceptors. It is a forced voluntary expiration of the subject against a resistance. It consists of four phases.

PhaseI- Increase in transthoracic pressure, transient increase in blood pressure and fall in heart rate.

Phase II- Reduced venous return resulting in low stroke volume, ultimately ends in reduced blood pressure and compensatory tachycardia.

Phase III- End of expiration resulting in further reduced blood pressure due to pulmonary vascular expansion and heart rate increases.

Phase IV–Baroreceptor activation, abrupt raise in blood pressure above the resting value and the concomitant bradycardia occurs.

Valsalva Ratio = longest RR interval/ shortest RR interval

Procedure:

The precautions are same as that of the previous test. The ECG should be recorded from the resting period throughout the procedure. The subject is asked to blow into a mercury manometer to maintain a column of mercury at 40mmHg for 15s. The results of the test relay on proper performance of the manoeuvre, age, sex, body positions and drugs.

4.7. Cold Pressor Test (CPT):

Immersion of hand or feet inside the cold water (4^0) for 60- 90 seconds cause activation of sympathetic system via afferent pain and temperature fibres of skin. There is a raise in diastolic blood pressure which is a measure of sympathetic activity.

Procedure:

All the precautions should be taken similar to the other tests. One hand of the subject is immersed inside the cold water. The blood pressure should be measured on the opposite hand after 90s of immersion. The result is presented as difference between the procedure blood pressure and the resting bloodpressure.

4.8 Isometric Hand Grip:

When the subject is isometrically pressing a hand grip dynamometer using $1/3^{rd}$ of maximal strength for 3-5 minutes, there is raise in diastolic pressure. The elevated diastolic pressure is due to cardiac acceleration without increase in peripheral vascular resistance.

Procedure:

After a period of rest the subject is asked to press the isometric hand grip upto the maximal power. Then the subject is instructed to maintain $1/3^{rd}$ of maximal capacity for three minutes and the raise in diastolic blood pressure is noted.

4.9 orthostatic test:

After a period of rest in supine position, the subject assumes an upright position, there occurs hemodynamic changes. These changes measure the activity of baroreceptors and ultimately measure the activity of both sympathetic and parasympathetic

activity. After assuming an upright position there is pooling of blood in the lower limbs, decrease in venous return and stroke volume and it initiates a compensatory mechanism.

Immediate response occurs in 1-2 min, abrupt fall in blood pressure and acceleration of heart rate.

The heart rate changes are measured at 30^{th} and the 15^{th} beat. There is an initial fall in heart rate at 15^{th} beat after standing and there is an increase at 30^{th} beat.

30/15= RR Interval at 30^{th} beat/ RR interval at 15^{th} beat

PROCEDURE:

After a period of rest around 10 minutes in supine positon, the subject is allowed to stand immediately without any support and using both the legs equally and the ECG changes are recorded.

ASSESSMENT OF SERUM VEGF:

Serum VEGF levels are assessed by VEGF ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human VEGF in serum. This assay employs an antibody specific for human VEGF coated on a 96-well plate. Standards and samples are pipetted

into the wells and VEGF present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human VEGF antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and colour develops in proportion to the amount of VEGF bound. The Stop Solution changes the colour from blue to yellow, and the intensity of the colour is measured at 450 nm.

Figure 4.1

MATERIALS REQUIRED:

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 litre graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions

ASSAY PROCEDURE:

1. Prepare all reagents, samples and standards as instructed.
1. 2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4^{o}C.
2. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.
3. Add 100 µlprepared Streptavidin solution. Incubate 45 minutes at room temperature.
4. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
5. Add 50µl Stop Solution to each well. Read at 450 nm immediately.

CALCULATION OF RESULTS:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

All the results are calculated and mean and standard deviation is estimated. The data is the statistically analysed by using one way ANOVA.

The correlation between the data is assessed by Pearson's correlation.

The data between the two groups are assessed by unpaired student's 't' test.

RESULTS

The data obtained from conducting the Autonomic Function Tests and serum VEGF were statistically analysed using the Statistical Package for Social Sciences (SPSS) software version 22. From the data, mean and standard deviation of the variables are determined for the individual three groups. One way ANOVA test was employed for statistical analysis.

*** 'P' value < 0.05 was considered as significant**

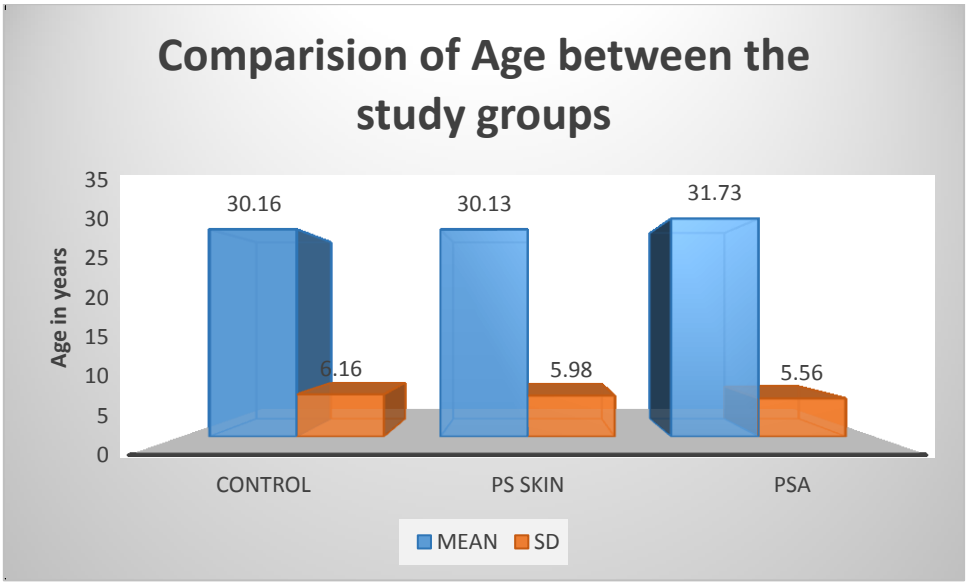
*** 'p' value < 0.01 was considered as highly significant**

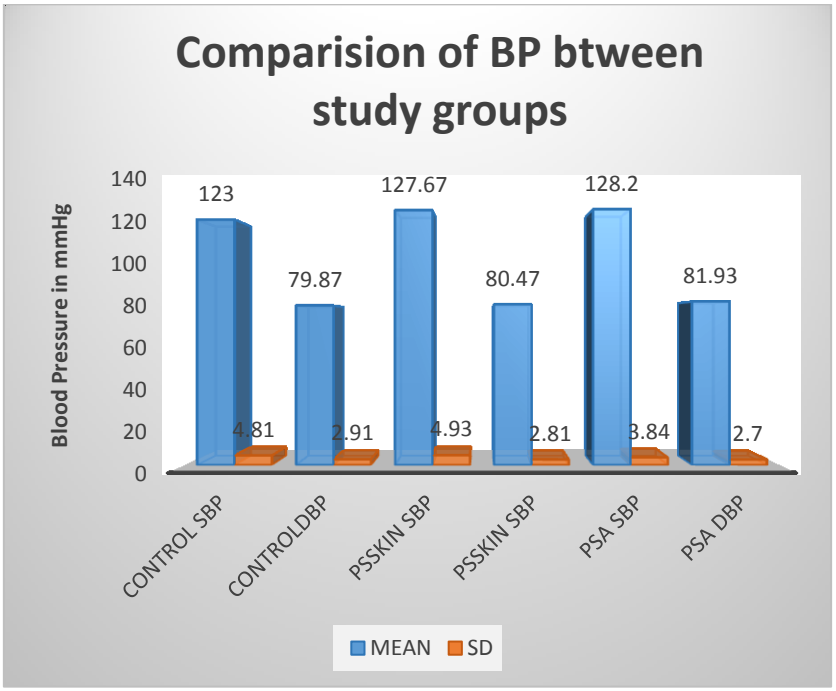
*** 'p' value < 0.001 was considered as very highly significant**

5.1 Comparison of Age

In three study groups, age is compared and the results along with ANOVA is shown in table 5.1. The mean age of control group is 30.17± 6.6, and that of Psoriatic skin patient's group and Psoriatic arthritis patient's group is 30.13±5.98, 31.13±5.56 respectively. The difference between the age group is found to be statistically not significant.

Study groups	Mean Age	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	30.17	6.6	**Between groups**	2	19.23	9.62	0.262	**0.77**
Psoriatic skin	30.13	5.98						
Psoriatic arthritis	31.13	5.56	**Within Groups**	87	3183.36	36.59		





5.2 Comparison of resting blood pressure:

The resting systolic and diastolic blood pressure of the study was measured and statistically analysed and the results are shown in table 5.2. The resting systolic blood pressure was found to be statistically highly significant (p value 0.001) and that of diastolic blood pressure is statistically not significant (p value 0.85)

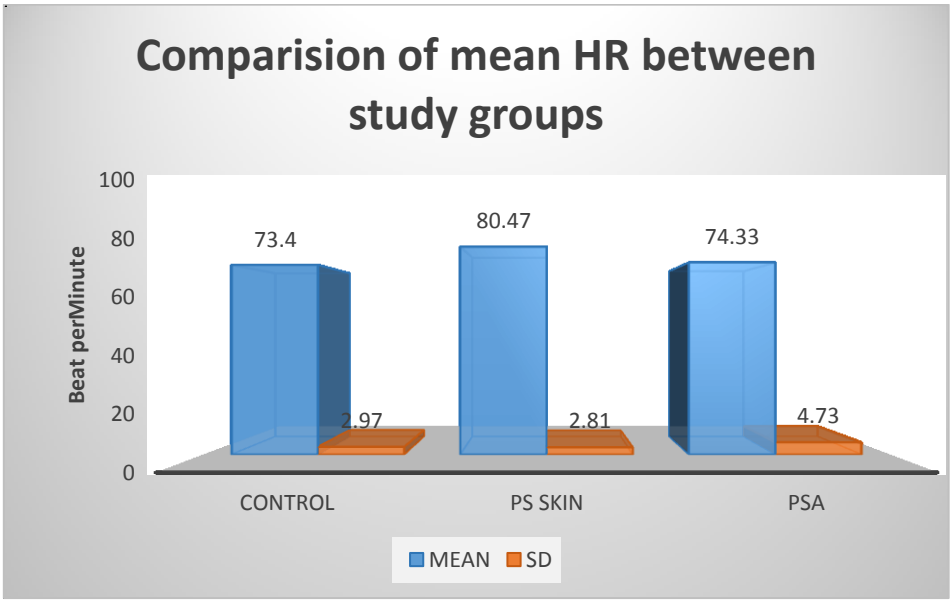
Table 5.2

Study groups	Mean S BP	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	123.2	481	**Between groups**	2	491.29	245.64	11.8	**0.001**
Psoriatic skin	127.67	4.93						
			Within Groups	87	1803.41	20.728		
Psoriatic arthritis	128.20	3.84						

Study groups	Mean D BP	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	79.05	2.97	**Between groups**	2	78.7	39.36	2.56	**0.85**
Psoriatic skin	79.73	2.96						
			Within groups	87	1336.63	15.36		
Psoriatic arthritis	81.43	5.56						

5.3 Comparison of Mean Heart Rate time domain variable:

The mean heart rate of the study groups is calculated from resting Heart rate variability statistically time domain analysis. The results are mentioned in



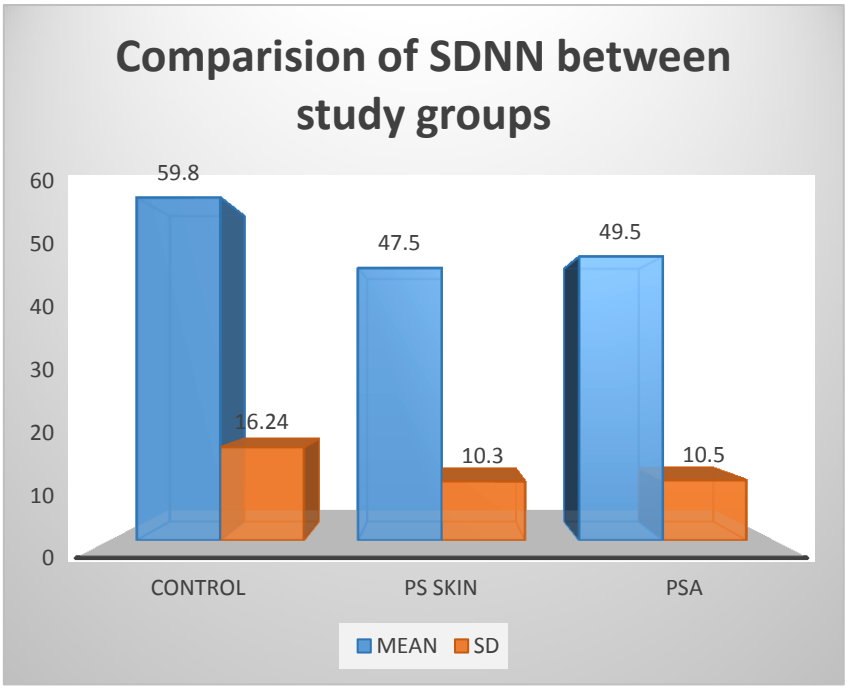


Table 5.3. The results show there is no statistical significance between the three groups and the 'p' value is **0.335**

Table5.3

Study groups	Mean HR	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	73.40	5.18	**Between groups**	2	45.75	22.88	1.10	**0.335**
Psoriatic skin	75.07	3.59						
			Within Groups	87	1800.71	20.70		
Psoriatic arthritis	74.33	4.73						

5.4 Comparison of SDNN values:

The time domain value SDNN for control is 59.80± 16.24, and that for skin and arthritis patients is 47.50± 10.30 and 49.50± 10.30 respectively. The statistical analyse of the data showed no clinical significance ('p' value 0.7)

Table 5.4

Study groups	Mean 30/15	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	59.80	16.2	**Between groups**	2	2613.8	1306.9	6.7	**0.7**
Psoriatic skin	47.50	10.3						
			Within Groups	87	16888.4	194.12		
Psoriatic arthritis	49.5	10.3						

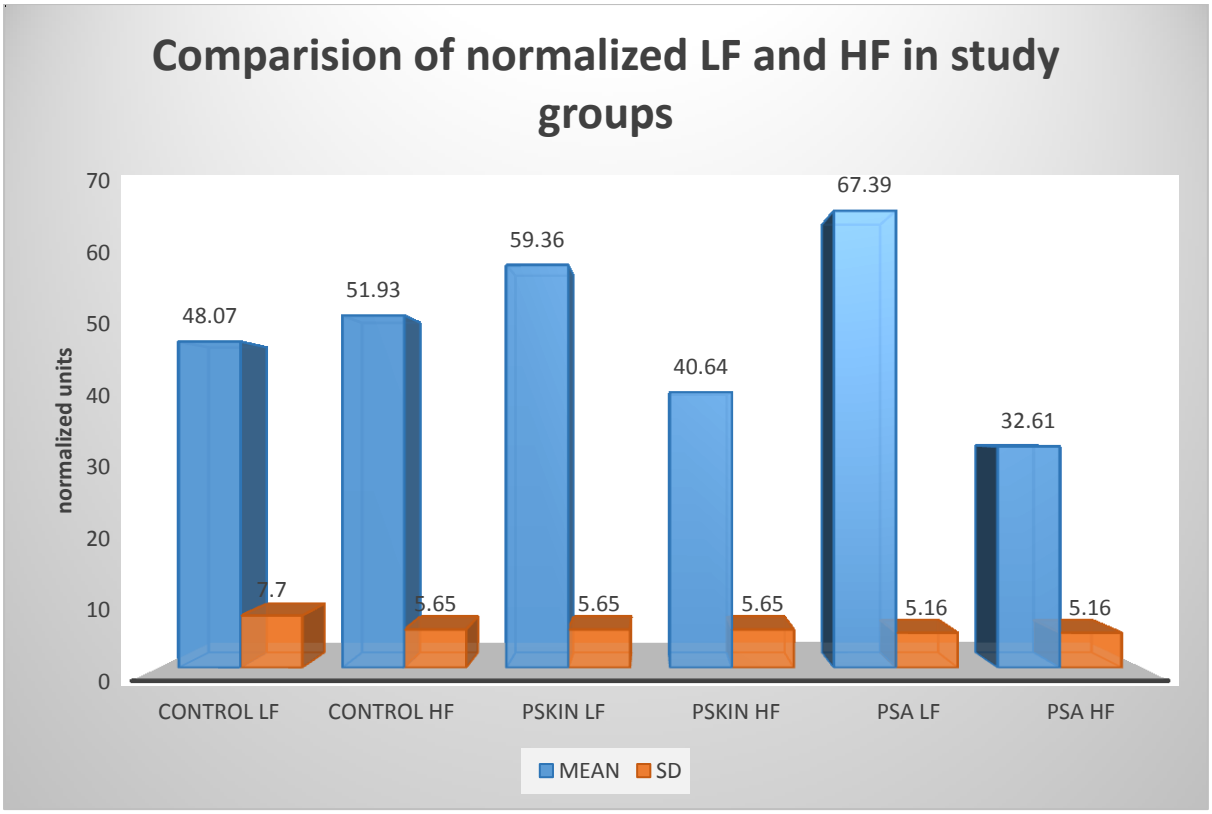
5.4 Comparison of resting Heart Rate Variability frequency domain variables:

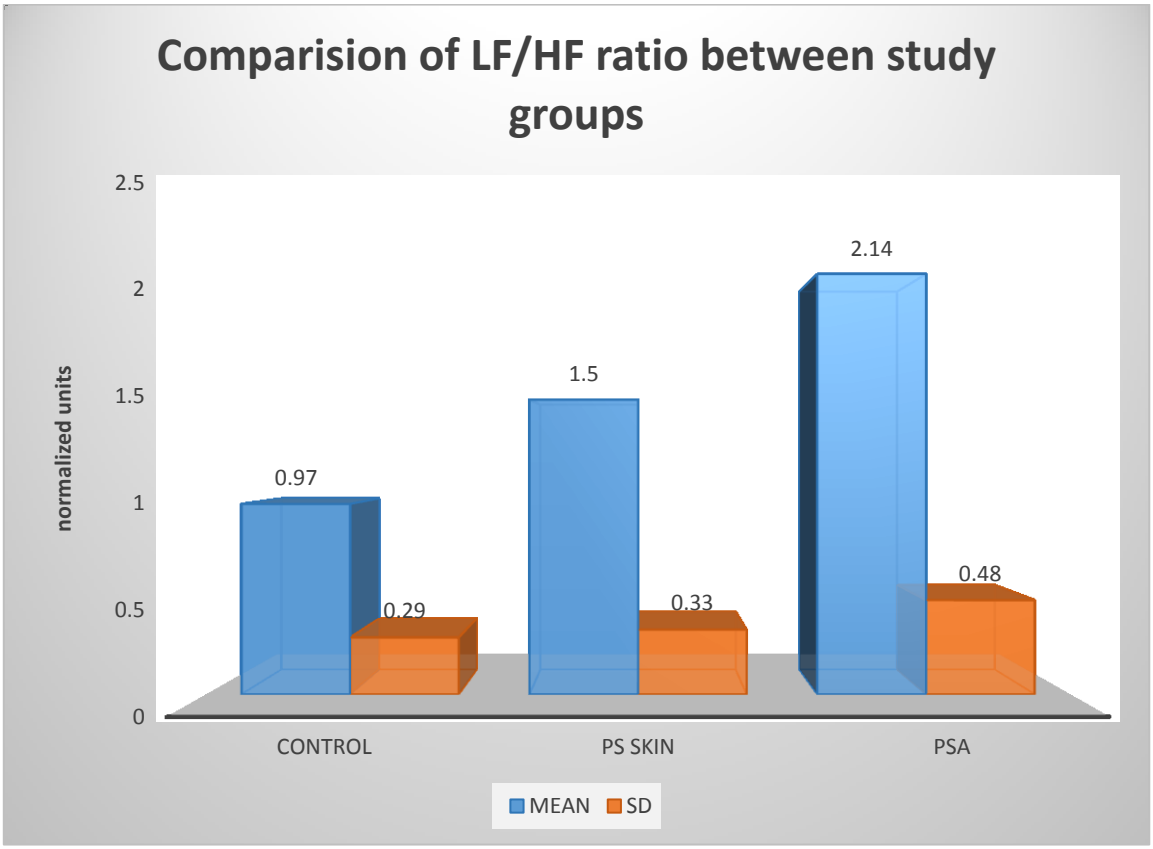
The frequency domain values of the study groups and their statistically significance was shown table 5.4. The LF normalised units (nu) which is a sympathetic tone indicator is higher in psoriatic skin and PsA patients. The HF normalised units an indicator of parasympathetic tone is significantly reduced in psoriatic skin and PsA patients. The ratio between LF and HF is an indicator of sympathovagal balance and it is significantly higher in the psoriatic skin and PsA patients suggesting an autonomic dysfunction.

Table5.5

Study groups	Mean nLF	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	48.07	7.70	**Between groups**	2	5652.07	2826	71.94	**0.000**
Psoriatic skin	59.36	5.16						
Psoriatic arthritis	67.39	5.65	**Within Groups**	87	3417.30	39.24		

Study groups	Mean n HF	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	51.93	7.7	**Between groups**	2	5652	2826	71.94	**0.000**
Psoriatic skin	40.64	5.65						
Psoriatic arthritis	32.61	5.16	**Within Groups**	87	3417.3	39.27		


Comparision of normalized LF and HF in study groups
normalized units
70
60
50
40
30
20
10
0
48.07
7.7
51.93
5.65
59.36
5.65
40.64
5.65
67.39
5.16
32.61
5.16
CONTROL LF
CONTROL HF
PSKIN LF
PSKIN HF
PSA LF
PSA HF
MEAN
SD


Comparision of LF/HF ratio between study groups
normalized units
2.5
2
1.5
1
0.5
0
0.97
0.29
1.5
0.33
2.14
0.48
CONTROL
PS SKIN
PSA
MEAN
SD

Study groups	Mean LF/HF	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	0.97	0.29	**Between groups**	2	20.60	10.29	72.95	**0.000**
Psoriatic skin	1.50	0.33						
			Within Groups	87	12.27	0.141		
Psoriatic arthritis	2.14	0.48						

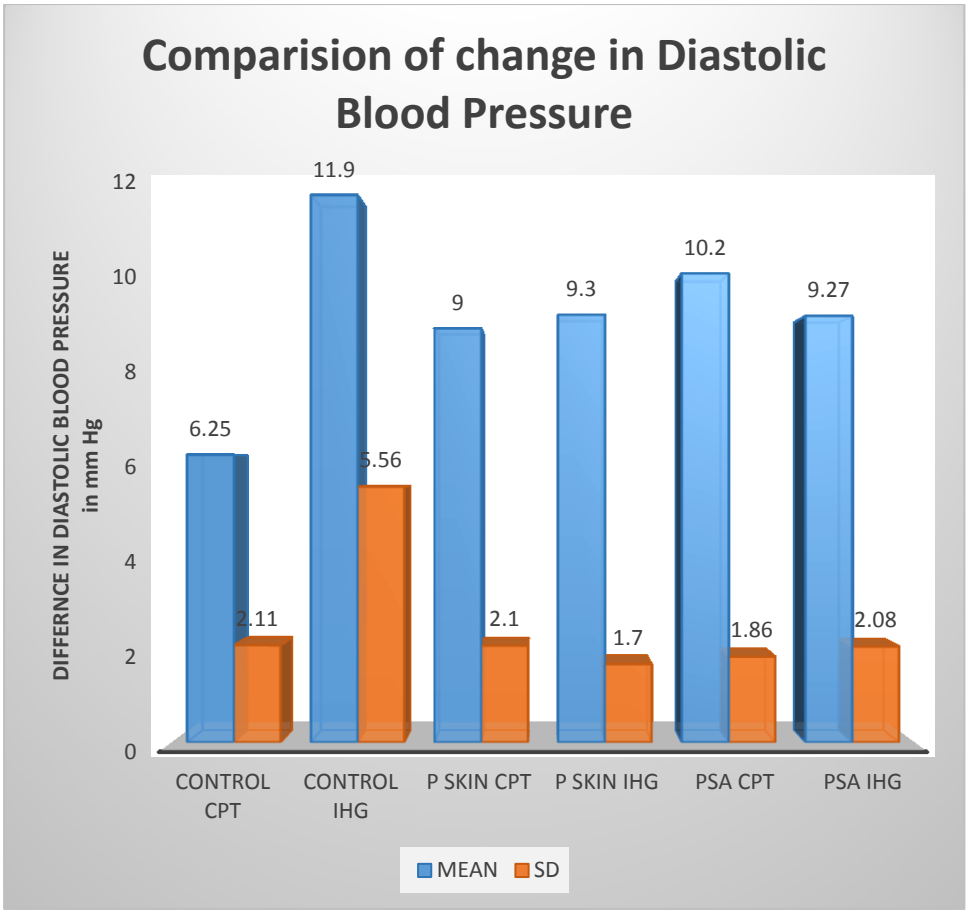
5.5 Comparison of autonomic function tests:

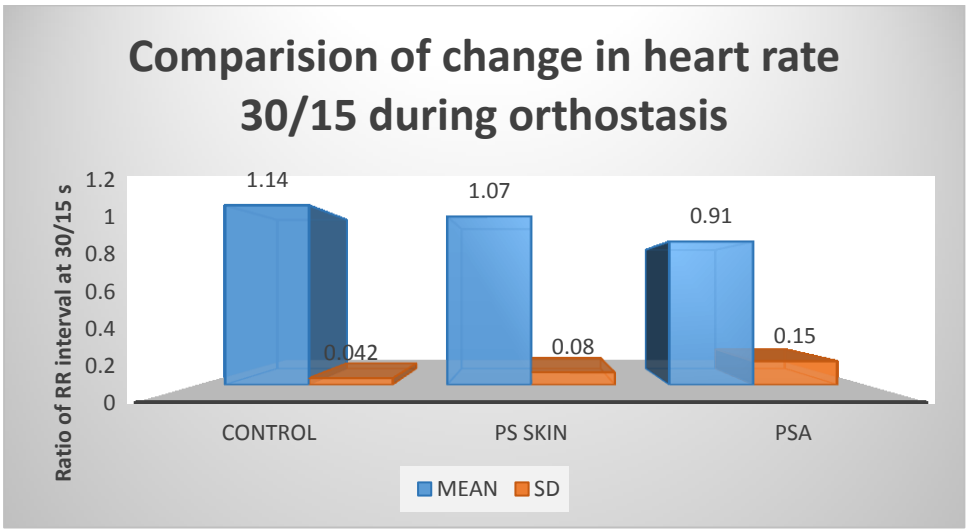
5.5.1 Orthostatic standing:

The mean ratio between the 30th heart beat RR interval and the 15th heart beat RR interval during orthostatic test between the study groups is calculated and statistically analysed and the results shows very high significance (table **5.5)**.

Table5.6

Study groups	Mean 30/15	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	1.14	0.04	**Between groups**	2	1.374	0.687	66.79	**0.000**
Psoriatic skin	1.07	0.08						
			Within Groups	87	0.8947	0.016		
Psoriatic arthritis	0.85	0.15						


Comparision of change in Diastolic Blood Pressure
DIFFERNCE IN DIASTOLIC BLOOD PRESSURE in mm Hg
12
10
8
6
4
2
0
6.25
2.11
11.9
5.56
9
2.1
9.3
1.7
10.2
1.86
9.27
2.08
CONTROL CPT
CONTROL IHG
P SKIN CPT
P SKIN IHG
PSA CPT
PSA IHG
MEAN
SD


Comparision of change in heart rate 30/15 during orthostasis
Ratio of RR interval at 30/15 s
1.2
1
0.8
0.6
0.4
0.2
0
1.14
0.042
1.07
0.08
0.91
0.15
CONTROL
PS SKIN
PSA
MEAN
SD

5.5.2Cold pressor test:

The diastolic pressure is measured at 1 minute and 5 minute after immersing hand in cold water at 10 C and the results are shown in table 5.6. The results are statistically analysed and found to very highly significant.

Table 5.7

Study groups	Mean 1mt DBP	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	6.56	2.11	**Between groups**	2	206.43	103.2	25	**0.000**
Psoriatic skin	9	2.1	**Within Groups**	87	357.3	4.1		
Psoriatic arthritis	10.2	1.86						

5.5.3.Isometric Hand Grip test:

The diastolic blood pressure is measured at 1minute and five minute after subjecting to 30% of maximum voluntary contraction to all the individuals in all the three study groups and the results were analysed statistically, show in 5.7. The result shows very high significance in one minute diastolic blood pressure ('p' value 003) and significant value in five minute diastolic blood pressure ('p' value 0.04).

Table 5.8

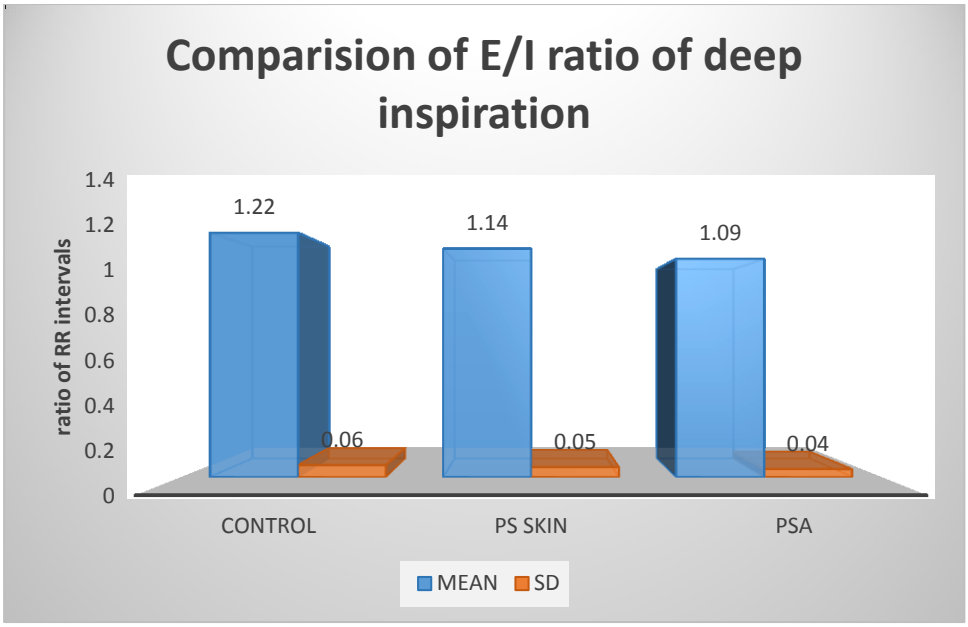
Study groups	Mean 1mt DBP	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control		2.13	**Between groups**	2	46.7	66.389	5.99	**0.003**
Psoriatic skin	9.30	1.7						
			Within Groups	87	338.89	3.90		
Psoriatic arthritis	9.27	2.08						

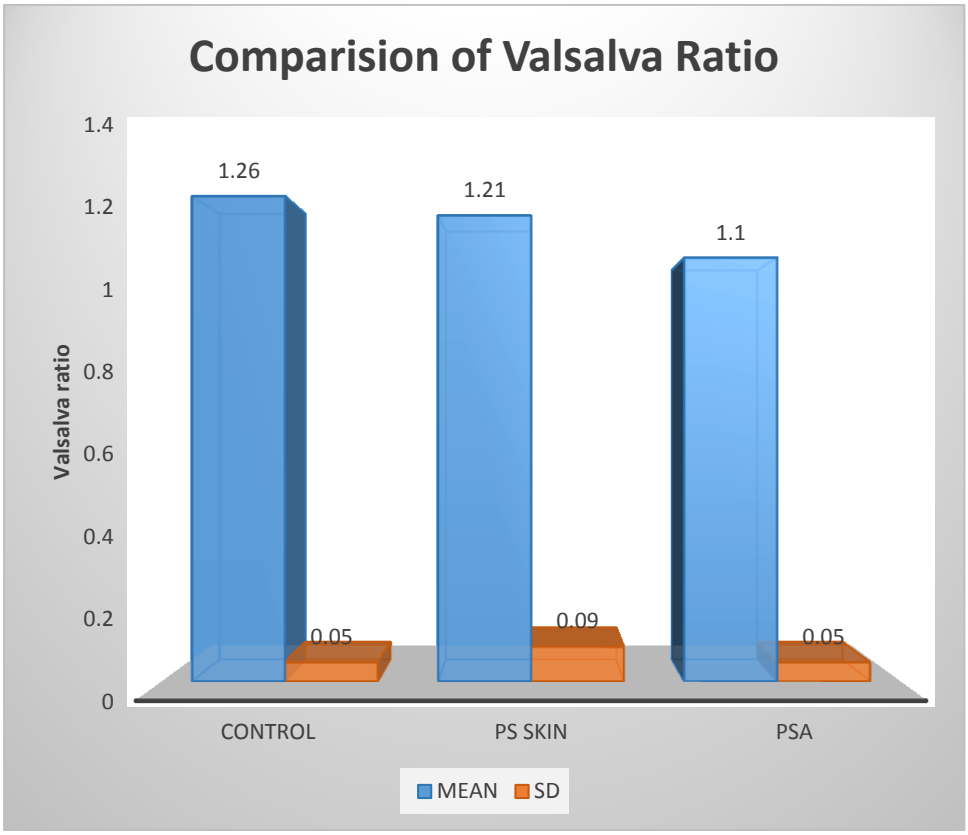
5.5.4.Deep Breathing:

The mean and standard deviation of E/I ratio of control group is 1.16±0.04 and that of psoriatic skin patients and PsA patients are 1.12±0.04 and 1.09±o.04 respectively (Table5.8). The results are statistically analysed using ANOVA and found to be statistically very highly significant ('p' 0.000)

Table 5.9

Study groups	Mean E / I	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	1.22	0.06	**Between groups**	2	0.258	0.129	50.25	**0.000**
Psoriatic skin	1.14	0.05						
			Within Groups	87	0.223	0.0026		
Psoriatic arthritis	1.09	0.04						


Comparision of E/I ratio of deep inspiration
ratio of RR intervals
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.22
0.06
1.14
0.05
1.09
0.04
CONTROL
PS SKIN
PSA
MEAN
SD


Comparision of Valsalva Ratio
Valsalva ratio
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.26
0.05
1.21
0.09
1.1
0.05
CONTROL
PS SKIN
PSA
MEAN
SD

5.5.5 Valsalva ratio:

The Valsalva ratio is calculated for the study groups and the mean and standard deviation for control is 1.26±0.05. The mean and standard deviation of psoriatic skin and PsA is found to be reduced than the control group and the values are 1.19±0.10 and 1.10±0.04 respectively. The values are statistically analysed and found to be very highly significant and shown in table 5.8.

Table 5.9

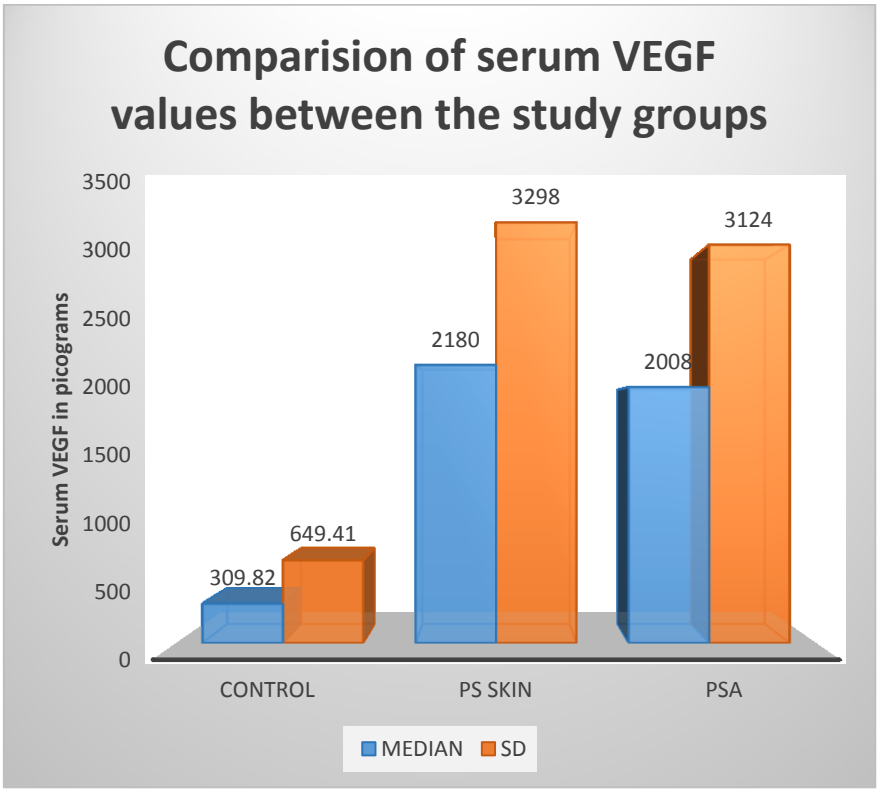
Study groups	Mean V/ R	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	1.26	0.05	**Between groups**	2	0.402	0.201	46.03	**0.000**
Psoriatic skin	1.21	0.09	**Within Groups**	87	0.38	0.043		
Psoriatic arthritis	1.10	0.05						

5.6 Serum VEGF:

The serum VEGF levels are found to be very high in psoriatic skin patient and PsA patient and the values in comparison with controls are found to statistically highly significant (table5.9)

Table 5.10

Study groups	Mean VEGF	SD	Source of variation	Df	Sum of squares	Mean square	F value	'p' value
Control	309.8	0.05	**Between groups**	2	64109700	32054900	4.56	**0.01**
Psoriatic skin	2180	3298						
			Within groups	87	610664000	7019130		
Psoriatic arthritis	2008	3124						



5.11Comparison of serum VEGF levels and age, resting blood pressure and mean heart rate and sympathovagal balance in control subjects:

Table 5.11.

VEGF	Age	Rest SBP	Rest DBP	Mean HR	nLF	nHF	nLF/nHF
R	-0.02	-0.1843	-0.267	-0.0204	0.191	-0.19	0.22

r – Pearson's correlation coefficient

The Pearson's correlation coefficient states that there is weak negative correlation between VEGF and the parameters age, resting blood pressure and mean heart rate shows slight negative correlation, which is not statistically significant in control subjects. The frequency domain values and serum VEGF shows weak correlation which is not statistically significant in control subjects.

5.12Comparison of serum VEGF levels and age, resting blood pressure and mean heart rate and sympathovagal balance in psoriatic skin patients:

Table 5.12.

VEGF	Age	Rest SBP	Rest DBP	Mean HR	nLF	nHF	nLF/nHF
R	-0.17	-0.112	0.0023	-0.0594	0.086	-0.086	0.0836

r – Pearson's correlation coefficient

The Pearson's correlation coefficient of psoriatic skin patient group states that there is very weak negative correlation between VEGF and the parameters age, resting systolic blood pressure and mean heart rate shows slight negative correlation, which is not statistically significant. The frequency domain values (nLF and LF/HF) and serum VEGF shows weak positive correlation which is not statistically significant in control subjects. The HF and serum VEGF shows weak negative correlation and again it is statistically not significant.

5.13 Comparison of serum VEGF levels and age, resting blood pressure and mean heart rate and sympathovagal balance in PsA patient group:

Table 5.13.

VEGF	Age	Rest SBP	Rest DBP	Mean HR	nLF	nHF	nLF/nHF
R	0.041	0.0368	0.0848	0.3239	0.129	-0.129	0.213

r – Pearson's correlation coefficient

The Pearson's correlation coefficient of PsA group states that there is very weak positive correlation between VEGF and the parameters age, resting blood pressure, mean heart rate, LF, and LF, HF ratio shows weak positive correlation, which is not statistically significant. The frequency domain values LF and serum VEGF

shows weak negative correlation which is not statistically significant PsA subjects.

Comparison of sympathovagal balance and serum VEGF between Psoriatic skin and PsA patients:

The normalized LF, HF ratio and serum VEGF values are statistically analysed by unpaired student's 't' test between Psoriatic skin and PsA patients and the results are shown in table 5.14. The LF, HF ratio shows high statistical significance ('p' value 0.000) and serum VEGF shows no significance ('p' value 0.8)

Table 5.14.

Variables	Mean ± SD		'p' value
	Psoriatic Skin	PsA	
LF / HF	1.50 ± 0.33	2.14 ± 0.48	**0.000***
Serum VEGF	2180.7± 3298	2008± 3124	**0.8**

DISCUSSION

The objective of the study is to assess the serum VEGF levels and evaluate the Autonomic Nervous System (ANS) in psoriatic skin and psoriatic arthritis patients and compare it between them. By assessing and comparing the ANS function and serum VEGF levels, the etiopathogenesis can be studied and the difference or similarity of the etiopathogenesis between the two forms of same disease can be identified. The integrity of sympathetic and parasympathetic components of ANS and the sympathovagal balance is assessed by standard tests like

- Resting HRV
- Orthostatic standing
- Cold pressor test
- Isometric hand grip
- Deep breathing
- Valsalva manoeuvre

6.1 Comparison of Age:

The age of individuals in all the three study group lies between 20-40 years to lighten the age related ANS changes. The mean age of control is 30.17± 6.6, and that of Psoriatic skin patient

and Psoriatic arthritis patient is 30.13±5.98, 31.13±5.56 respectively. The variability measured from the data between the groups showed variance and that is not due to the difference in age between the groups. One way ANOVA of age data between the three groups shows no statistical significance ('p' value 0.77).

6.2 Comparison of sex:

Psoriatic skin disease shows no sexual predilection and the male female ratio is 1:1(Parisi R, et al)[77].The subtypes of PsA may show slight male or female predilection, but as a whole the prevalence of the disease is equal in male and female similar to the skin disease(Prasad P etal)[76] . In order to avoid bias due to sex variation of ANS functions and serum VEGF levels, equal number of male and female subjects are taken for the study. Each study group contains 15 male and 15 female individuals.

6.3 Comparison of resting blood pressure:

Those individuals who are a known hypertensive, are not included in the study since the hypertensive subject shows altered ANS functions.(Julius .s et al)[77] and elevated serum VEGF levels. The resting systolic and diastolic blood pressure data is collected from the study groups. Though the mean systolic blood pressure lies

within normal range (120-140mmHg) in all the three groups, the observed value shows increased systolic pressure in both psoriatic skin (127.67± 4.81) and PsA (128.20± 3.84) than controls(123±4.81). The difference is also statistically highly significant, 'p' value is 0.001. Similarly the resting diastolic blood pressure is higher in psoriatic skin (80.47±2.81) and in PsA patients (81.93± 2.70) than controls (79.87±2.97). Though the difference is not statistically significant ('p' value 0.85) there is an elevation of diastolic blood pressure also.

In psoriatic skin disease there are series of linked cellular changes in skin which may be systemic in due course of time, and these changes are due T cell mediated autoimmune disease (J Krueger et al)[79]. The pathogenesis of PsA is similar to psoriatic skin disease (Oliver FitzGerald et al)[80]. Both the disease is a T cell mediated autoimmune disease primarily affecting the skin or musculoskeletal structures or both. The autoimmune disease leads to endothelial dysfunction (De Simone C, et al)[81]. The main cytokine behind it is the TNF factor released from T cells and other activated immune cells during inflammation (B. J. Nickoloff et al)[82].

The endothelial dysfunction is the shared pathogenesis between autoimmune disease and atherosclerosis. Endothelial cells

release both vascular contracting and vascular relaxing factors and the loss of balance occurs in endothelial dysfunction which results in hypertension (Brezinski EA et al)[83]. Though the study has excluded hypertensive patients the resting blood pressure of psoriatic skin and PsA is elevated than the normal controls, which eventfully ends in hypertension in due course of disease. Thus both psoriasis skin and psoriatic arthritis patients are in more risk to develop hypertension (Abrar A. and AhmetBacaksiz et al)[84, 85].

6.4 Comparison of resting HRV time domain values:

The mean Heart Rate of the three groups is calculated for the 5 minutes during the recording of resting HRV and the values are not statistically significant. Similarly the SDNN values of the three groups also shows no statistical significance. The mean HR and SDNN is ill –defined statistical quantity, because they are dependent on the duration of recording period. Thus it is inappropriate to compare short term mean HR and SDNN.

Asuman Bicer et al[86] showed that SDNN and mean HR showed no significance between control and psoriatic skin patients. Similar results are obtained by Haligur BD et al[87] also showed similar results in short term resting HRV. Similar results are shown in PsA

patients by Oliver Fitzgerald et al[80]. The time domain parameters mean Heart rate and SDNN yields better results in 24 hour Holter monitoring than short term resting HRV monitoring(Saul JP et al)[89].

6.5 Comparison of frequency domain parameters of short term HRV:

A highly significant variation in frequency domain variables is observed in this study. The LF and HF values are altered by the total power changes. To minimise this effect normalized values are taken. In this study the nLF value is raised in psoriatic skin and PsA patients. The nLF value for psoriatic skin and PsA patients are 59.36± 5.36 and 67.39± 5.16 respectively, which is higher than that of control mean 48.07± 7.7. The results on statistical analysis show very high significance ('p' value 0.000).

The nLF component in frequency domain is considered as a marker of sympathetic nervous system modulator (MallaniA et al)[90]. Akserlord S et al[91] considers the component as a parameter having both parasympathetic (vagal) and sympathetic influences. Asuman Bicer et al[86] study have showed a significant increase in nLF similar to this study and states that there is autonomic dysfunction in psoriasis and it is associated with increased sympathetic component. Schmidt et al[92] stated that in their study there is no significant

difference between LF component between control and Psoriasis skin group.

It is an established fact that skin is a part of neuro – endocrine and immune organ whose functions are governed by central regulatory systems (Barland et al)[93]. The concentration of β 2 receptors in skin contribute to epidermal homeostasis and it is partly governed the ANS and immune system. The release of neurotransmitter from adrenergic nerve terminals regulates the magnitude of adaptive immune responses. The immune cells are governed locally as well as systemically by the catecholamines via the expression of adrenergic receptors (Schallreuter KU et al)[94].It is observed that in psoriasis there is an elevated level of circulating catecholamines, down regulation of β2 receptors and increased levels of the epinephrine synthase enzyme[61]. Thus in psoriasis there is an over production of catecholamine and its failure to control innate immunity ultimately leading to sympathetic over activity.

Though the pathogenesis of PsA is similar to skin disease PsA has a more systemic involvement than skin disease alone. Syngle A[95] et al showed that in PsA both sympathetic and parasympathetic dysfunction occurs. Though Rheumatoid arthritis and PsA are different entities, they share some common pathophysiological course. Increased nLF is observed in Rheumatoid arthritis,

suggesting elevated cardiac sympathetic activity and the etiology is said to inflammatory stress caused by the activated autoimmune cells and he cytokines released by them (Dekkers JC et al)[97]. Thus the cause of elevated nLF in this study is result of cardiac sympathetic over activity caused by inflammatory stress. The circulating levels of catecholamine and down regulation of β receptors also occur in PsA.

The nHF component of frequency domain spectral analysis of HRV is considered as a major contributor of efferent vagal activity. In this study the nHF mean value of psoriatic skin (40.64± 5.65) and PsA (32.61± 5.16) is reduced than that of controls (51.93±7.7). The reduced value of nHF states that there is reduced parasympathetic activity in psoriatic skin and PsA. In PsA the para sympathetic activity is still more reduced than skin involvement alone suggesting more severe and systemic involvement of PsA. The reduced parasympathetic activity in the presence of enhanced sympathetic activity reflected by increased nLF is suggested as parasympathetic withdrawal (Lawrence Wilson)[98.]

The nHF value suggest that the parasympathetic withdrawal is more prominent in PsA compared to psoriatic skin. This observation is supported by the works of Syngle A[95] et al suggesting that in PsA in there is more of para sympathetic dysfunction in comparison to

sympathetic over activity. The study of Syngle A et al is 24 hour ECG spectral analysis by which the power spectrum of LF component taken as an account of parasympathetic activity which is reduced in their observation. To conform para sympathetic dysfunction further ANS study is needed to confirm it. The mean nHF of psoriatic skin patients is also reduced in comparison to control groups suggesting parasympathetic dysfunction. AsumanBicer et al[86] study suggested than in psoriasis the LF component is reduced in comparison to controls suggesting a parasympathetic dysfunction.

The nLF/nHF ratio is considered as mirror of sympathovagal balance. Thus sympathetic over activity or parasympathetic reduction (withdrawal), increase the ratio. In this study the ratio is increased in psoriatic skin (1.50±0.33) and PsA (2.14±0.48) in comparison to controls (0.97± 0.27) and this difference is found to be statistically highly significant. The increased ratio suggests dysregulation of sympathovagal balance. Syngle A et al in their study both sympathetic and parasympathetic dysfunction suggesting loss of sympathovagal balance in PsA. Haligur, Beyzan et al[98] in their study suggested an increased sympathetic over activity and a normal parasympathetic functions in psoriasis. In AsumanBiçer et al[86] studies suggested loss of sympathovagal balance in psoriasis.

Psoriasis is a disease showing a wide spectrum of clinical manifestations including skin and musculoskeletal manifestations. The extent of disease depends on genetic inheritance, environmental factors like season, infections, stress, and also the response to treatment. Those who have severe clinical manifestation may shows severe loss of sympathovagalbalance.The factor in assessment of the sympathovagal balance is the duration of disease.

6.6 Comparison of Autonomic Functions:

Orthostatic standing

After a considerable time of rest the test subjects stand with both the legs equally balanced and the ratio of RR interval at 30^{th} beat and 15^{th} beat is calculated for all the individuals in all the groups. On assuming an upright position there is pooling of blood in extremities and reduced venous return resulting in reduced stroke volume. During this period there is acceleration of heart rate, which occurs during the first 15 seconds. To maintain hemodynamic parameters there occurs a compensatory reaction of raise in blood pressure within 30 seconds during which there is reduction of heart rate. Hence the ratio of RR interval during 30^{th} second and 15^{th} second is a measure of parasympathetic activity[99].

In this study the mean 30/15 of the control groups are calculated and reduced to 1.07± 0.04 in Psoriatic skin and 0.85 ± 0.15 in PsA patients. The values are tend to be lower than controls (1.14± 0.04). The 30/15 is a measure of activity of baroreceptor function and reflects the intactness of vagal reflexes. In both form of psoriasis the 30/15 value is reduced, but in comparison to skin disease in PsA it is much more reduced. Syngle A et al[95] in their study states parasympathetic disorder is most common in PsA than sympathetic dysfunction. In their study abnormal pseudo motor dysfunctions are reported. The etiology may be small fibre peripheral neuropathy associated with autoimmunity.

Cholinergic innervation of sympathetic sweat glands whose response is reduced in PsA. Similar results are also found in Rheumatoid arthritis and showed improvement of pseudo motor functions after treatment with Disease Modifying Drugs. Haligur et al in their study showed normal parasympathetic functions. In this study the deviation of 30/15 ratio of psoriatic skin patients is less in comparison to PsA.

Cold pressor test:

CPT is a test to access the sympathetic system, in which immersion of hands or feet in cold water around 4-6o C for 60- 90

seconds leads to activation of afferent temperature, pain from skin. The sympathetic activation causes increase in diastolic blood pressure without much alteration in peripheral resistance.

In this study it the diastolic blood pressure in one minute shows an increase in both psoriatic skin (9.0±2.1) and PsA patients (10.20±1.86). The raise in diastolic blood pressure in controls is 6.56± 2.11. The difference in blood pressure is found to be statistically highly significant ('p' value 0.000). The elevated diastolic blood pressure is due to increased outflow of impulses from sympathetic nervous system due to stimulation of pain and cold receptors. Syngle A et al showed increased sympathetic activity in PsA patients and Markuszeski et al also showed increased sympathetic activity in psoriatic skin patients resulting in elevated diastolic pressure in CPT.

***Isometric Hand Grip:**

In this study there is increased raise of diastolic blood pressure in psoriatic skin and PsA patients and the raise is also statistically significant. The reason for this elevation is similar to that of CPT, and the same explanation carries well. The raise of this diastolic blood pressure is associated with no alteration in

peripheral vascular resistance along with accelerated heart rate. The same results are attained by Syngle A et al[95] , Haligur B D et al[87].

***Deep breathing:**

Respiratory arrhythmia is the principle behind this test. During inspiration the heart rate increases and the reverse occurs in expiration. The ratio between the longest RR interval during expiration and the shortest duration during inspiration is calculated between the groups. The values are reduced in psoriatic skin and PsA patients and the reduction is said to statistically significant. Syngle A et al states in the study conducted on both PsA and RA suggests parasympathetic dysfunction in form of parasympathetic withdrawal. Similarly

Haligur et al states that psoriatic skin disease shows parasympathetic withdrawal and, AsumanBicer et al showed proper parasympathetic functioning in psoriasis. The AsumanBicer et al study includes deep breathing test and found to be reduced than control but the results are statistically not significant.

***Valsalva Ratio:**

The function of baroreceptors is evaluated by the Valsalva manoeuvre. The VR ration is longest RR interval in phase IV and the shortest interval between the phase II.

The VR ratio is reduced in both psoriatic skin and PsA patients than the controls and the result is found to be statistically significant. Valsalva ratio involves both sympathetic and parasympathetic components and the results can be interpreted as autonomic dysfunction. It cannot specify which component has gone wrong since the change in blood pressure is not measured. To measure blood pressure accurately in the four phases of the manoeuvre is difficult manually by sphygmomanometer.

6.7 Serum VEGF levels:

The serum VEGF levels (in picograms) the skin (2180±32) and PsA (2008±3128) patients are found to be highly elevated than in controls (309± 0.05) and the result is found to be statistically significant. There are many studies to support the elevated level of serum VEGF in skin lesions and also in the serum (Bhushan M et al[100], Creamer D[101]). It is the major epidermis derived growth factor specific for vessel growth (Detmar M)[102]. It is stated that the presence of altered VEGF gene provides strong suspicion to develop psoriasis (Young HS).

The level of VEGF is also elevated in PsA patients and the result is supported by Przepiera- Bedzak H et a [103].It is stated in that study that VEGF plays an important role in pathogenesis of the

disease. It is also stated that VEGF levels reflect the disease activity[104].

In this study there is variation of values between the patient groups and the variations are to level of severity of the disease and the heterogeneity nature of the disease.

6.8Correlation of serum VEGF levels and autonomic activity:

The serum VEGF levels are weakly associated with autonomic functions in all the three study groups. Thus serum VEGF levels has little or no influence in the autonomic functions. They reflect the severity, extent of involvement, and also the response to treatment (Flisiak I et al)[105]. Autonomic dysfunction is an ongoing process may take many years to evolve. Thus the levels of autonomic function cannot be accessed by the serum VEGF levels.

6.9 Comparison of serum VEGF levels between psoriasis skin and arthritis:

The serum VEGF levels are elevated in both the disease groups and inter comparison between them using unpaired student's 't' test shows no statistical significance ('p' value 0.8). Thus serum VEGF levels are elevated in both the disease and there is no difference between the values.

6.10 Comparison of ANS function between Psoriatic skin and Psoriatic arthritis:

The LF/HF ratio is taken to access the sympathovagal balance the study group. The results shows high statistical significance. In PsA there may be both skin and musculoskeletal involvement. PsA is also considered as severe form of disease and shows manifestation in patients having severe skin involvement. However all PsA patients on a routine do not show severe skin manifestation clinically because of the systemic treatment taken for musculoskeletal disease heals the skin lesions in due course of time. Thus it is concluded that PsA is a severe form systemic disease. Both the form of disease share some same etiology and pathogenesis. Hence the ANS dysfunction is severe in PsA patients than that of psoriatic skin disease alone.

LIMITATIONS OF THE STUDY

1. The study group is small containing only 30 individuals. The sample size is too small to substantiate the results.
2. The patients in both groups are not inter classified according to severity, and treatment wise.
3. The psoriatic skin patient may develop into PsA and a longitudinal study can assess the progression ANS dysfunction in them.
4. The role of ANS functions in such transition is not studied.
5. The role of duration of the disease in ANS is not defined.
6. The wide variation of serum VEGF levels in the disease groups is not proved to be due to severity, the heterogeneity of the disease or due to the effect of treatment.
7. The modification of ANS functions in response to treatment is not studied in this study. A longitudinal study may evaluate it.

CONCLUSION

This study states that in both psoriatic skin and PsA patients there is reduced HRV with sympathetic over activity and parasympathetic withdrawal and loss of sympathovagal balance. The severity of the ANS dysfunction is more in PsA. In both the disease types sympathetic over activity predominates.

The serum VEGF levels are elevated in both psoriatic skin and PsA patients. The serum VEGF levels shows no difference between the disease groups.

The levels of serum VEGF has no influence over the autonomic functions of the patients, but acts as a inflammatory marker and reflects the severity of disease.

The autonomic function tests helps to determine the autonomic dysfunctions earlier even before the onset of clinical findings. The control of disease in form of reduced inflammation may reduce the progression of ANS dysfunction and definitely reduce the serum VEGF levels.

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INFORMED CONSENT FORM

Title of the study: A comparative study of autonomic dysfunction and serum levels of Vascular Endothelial Growth Factor in Psoriasis skin disease and Psoriatic arthritis patients

Name of the Participant:

Name of the Principal Investigator: Dr.I. Kanagashree

Name of the Institution:

Institute of Physiology and Experimental Medicine,

Madras Medical College and Govt. General Hospital,

Chennai - 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in A comparative study of autonomic dysfunction and serum levels of Vascular Endothelial Growth Factor in Psoriasis skin disease and Psoriatic arthritis patients

1. I have read and understood this consent form and the information provided to me.

2. I have had the consent document explained to me.

3. I have been explained about the nature of the study.

4. I have been explained about my rights and responsibilities by the investigator.

5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.

6. I have been advised about the risks associated with my participation in this study.

7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.

8. I have not participated in any research study within the past _____month(s).

9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.

10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.

12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

13. I have understand that my identity will be kept confidential if my data are publicly presented.

14. I have had my questions answered to my satisfaction.

15. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____

Date_____

PROFORMA

1. Name :
2. Age:
3. Sex:
4. Address :
5. Occupation :
6. Complaints/duration:
7. Psoriasis Area and Severity Index
8. History and duration of joints involvement
9. Other associated symptoms-
10. Past history:
11. History of any drug intake
12. History of associated illness:
    a. Diabetes
    b. Hypertension
    c. Ischemic heart disease
    d. Respiratory diseases
    e. Hypothyroidism

EXAMINATION

General examination:

Pulse rate:

Blood pressure:

Systemic examination:

Cardiovascular system:

Respiratory system:

Gastrointestinal system:

Central nervous system:

CONTROL DATA

S. No	AGE	SEX	Rest SBP	REST DBP	MEAN HR	SDNN	nu LF	N u HF	LF/HF	OST 30/15	CPT DBP	IHGT DBP	E/I	VR	VEGF
1	28	F	120	76	67	69	34.5	65.5	0.53	1.23	6.8	16	1.24	1.24	7.6
2	35	F	126	82	78	55	43.2	56.8	0.76	1.13	7.8	22	1.19	1.21	538
3	35	F	120	80	69	65	48.3	51.7	0.93	1.13	8	24	1.24	1.29	2.5
4	23	F	118	72	70	50	48.6	51.4	0.95	1.19	4	18	1.21	1.21	4.9
5	30	F	120	80	75	52	55.4	44.6	1.24	1.16	4.6	12	1.16	1.25	7.5
6	24	F	120	74	78	48	49.5	50.5	0.98	1.18	8.2	14	1.22	1.32	4.6
7	35	F	118	80	64	50	58.4	41.6	1.40	1.07	6	16	1.28	1.29	11.2
8	25	F	120	78	71	91	34.9	65.1	0.54	1.15	4.2	10	1.22	1.27	120
9	26	F	124	78	76	51	55.8	44.2	1.26	1.2	6.2	14	1.2	1.33	2246
10	24	F	120	80	77	91	39.3	60.7	0.65	1.09	6.8	12	1.22	1.21	46
11	39	F	124	80	74	49	56.4	43.6	1.29	1.15	6.4	16	1.22	1.19	7.9
12	40	F	124	80	78	91	52.3	47.7	1.10	1.19	8	10	1.19	1.23	7.5
13	32	F	134	78	69	51	45.4	54.6	0.83	1.12	4	16	1.34	1.32	67
14	21	F	116	74	74	94	43.4	56.6	0.77	1.1	6	20	1.32	1.26	2234
15	24	F	118	82	81	61	38.5	61.5	0.63	1.08	14	21	1.21	1.24	123
16	22	M	120	80	87	37	48.4	51.6	0.94	1.15	6	10	1.24	1.32	7.9
17	22	M	120	84	83	52	43.4	56.6	0.77	1.12	8	9	1.12	1.21	7.6
18	23	M	120	80	72	54	45.8	54.2	0.85	1.12	4	8	1.14	1.21	34
19	28	M	126	84	74	55	56.6	43.4	1.30	1.06	4.8	9	1.19	1.39	12.8
20	36	M	130	84	69	65	54.4	45.6	1.19	1.12	6.4	10	1.24	1.25	73.7
21	40	M	134	82	65	83	55.5	44.5	1.25	1.14	4	8	1.24	1.23	76
22	24	M	120	80	68	51	61.4	38.6	1.59	1.08	8	7	1.22	1.31	2000
23	33	M	120	84	73	65	43.3	56.7	0.76	1.18	6.4	10	1.16	1.21	120
24	36	M	124	80	74	37	43.8	56.2	0.78	1.12	8	4	1.12	1.23	80
25	33	M	120	84	70	65	48.4	51.6	0.94	1.16	8.4	8	1.24	1.27	7.8
26	36	M	122	80	74	36	51.2	48.8	1.05	1.16	4	4	1.2	1.26	46
27	32	M	128	80	72	65	34.9	65.1	0.54	1.12	6.4	6	1.34	1.32	6.3
28	32	M	124	80	72	46	56.4	43.6	1.29	1.18	8.2	9	1.24	1.21	89
29	40	M	130	80	78	65	56.4	43.6	1.29	1.09	4.8	8	1.24	1.26	7.8
30	27	M	130	80	70	50	38.4	61.6	0.62	1.16	8.4	6	1.32	1.26	98

PSORATIC SKIN DATA

S. No	AGE	SEX	RESTSBP	REST DSP	REST HR	SDNN	NU LF skin	Nu HF	LF/HF	30/15	CPT DBP 1	IHG DBP1	E/I R	VR Ratio	VEGF
1	25	F	124	80	72	42	57.2	42.8	1.34	1.11	11	10	1.18	1.32	146
2	32	F	130	82	78	46	56.5	43.5	1.30	1.13	9	9	1.08	1.22	189
3	28	F	130	80	74	38	56.4	43.6	1.29	1.08	8	9	1.14	1.1	230
4	24	F	124	80	73	46	48.6	51.4	0.95	1.14	6	5	1.2	1.25	6000
5	34	F	140	80	76	47	65.5	34.5	1.90	1.03	15	12	1.13	1.43	178
6	23	F	120	80	78	45	58.4	41.6	1.40	1.05	11	9	1.2	1.09	234
7	21	F	120	70	72	39	55.5	44.5	1.25	1.1	9	6	1.11	1.21	125
8	23	F	128	78	80	49	64	36	1.78	1.13	8	8	1.12	1.12	245
9	38	F	134	80	72	69	59.7	40.3	1.48	1.02	9	11	1.16	1.24	6000
10	32	F	130	84	78	54	62.3	37.7	1.65	1.01	9	10	1.12	1.12	1234
11	40	F	130	80	71	58	64.3	35.7	1.80	1.03	11	9	1.18	1.26	6000
12	24	F	124	76	68	37	44.7	55.3	0.81	0.9	8	9	1.21	1.22	156
13	28	F	130	80	73	68	55.8	44.2	1.26	1.12	10	10	1.24	1.12	168
14	38	F	126	82	76	48	62.3	37.7	1.65	1.16	6	7	1.15	1.26	95
15	31	F	132	80	77	65	58.4	41.6	1.40	1.1	11	10	1.15	1.32	156
16	32	M	130	86	73	43	59.8	40.2	1.49	1.15	12	11	1.09	1.24	56
17	24	M	120	82	74	38	64.3	35.7	1.80	1.13	10	9	1.15	1.1	20144
18	33	M	128	80	76	64	59.6	40.4	1.48	1.12	8	12	1.13	1.4	6000
19	24	M	128	80	72	38	67.5	32.5	2.08	1.03	10	11	1.22	1.16	126
20	23	M	124	84	78	58	62.8	37.2	1.69	1.01	5	12	1.07	1.14	6000
21	40	M	132	80	72	65	66.7	33.3	2.00	1.08	7	8	1.13	1.24	59
22	33	M	126	80	76	43	49.8	50.2	0.99	1.06	11	11	1.14	1.21	134
23	30	M	124	80	82	39	52.4	47.6	1.10	1.1	7	8	1.24	1.18	129
24	22	M	124	84	79	36	53.8	46.2	1.16	1.13	9	10	1.08	1.12	6000
25	36	M	138	82	76	44	66.4	33.6	1.98	1.06	10	10	1.11	1.13	211
26	40	M	128	80	80	48	58.4	41.6	1.40	1.07	7	8	1.06	1.21	54
27	37	M	130	84	67	36	64.2	35.8	1.79	1.1	8	9	1.08	1.12	145
28	29	M	120	80	74	37	58.8	41.2	1.43	0.8	11	10	1.03	1.24	111
29	32	M	130	80	75	43	61.2	38.8	1.58	1.12	6	7	1.12	1.21	123
30	28	M	126	80	80	42	65.5	34.5	1.90	0.9	8	9	1.07	1.26	112

PSORATIC ARTHRITIS DATA

S. No	AGE	SEX	RESTSBP	REST DSP	REST HR	SDNN	Nu LF	Nu HF	LF/HF	30/15	CPT DBP 1	IHG DBP1	E/I R	VR	VEGF
1	34	F	128	84	76	38	68.4	31.6	2.16	1.04	10	8	1.11	1.13	6000
2	33	F	126	78	69	47	68.5	31.5	2.17	1.02	12	12	1.14	1.16	123
3	22	F	126	78	69	48	73.4	26.6	2.76	1.03	11	10	1.1	1.08	2344
4	33	F	130	80	76	36	69.6	30.4	2.29	1.12	13	8	1.12	1.14	324
5	34	F	128	80	73	63	71.4	28.6	2.50	0.97	9	8	1.11	1.14	142
6	23	F	120	80	72	54	67.8	32.2	2.11	0.9	10	11	1.08	1.1	146
7	35	F	128	86	76	36	75.4	24.6	3.07	0.93	9	4	1.07	1.02	6000
8	24	F	124	82	79	65	69.4	30.6	2.27	0.78	8	6	1.03	1.03	2345
9	33	F	128	86	73	46	69.4	30.6	2.27	1.08	9	11	1.1	1.18	212
10	38	F	128	84	79	42	69.4	30.6	2.27	0.87	10	14	1.04	1.11	1546
11	25	F	128	86	81	49	62.2	37.8	1.65	1.06	11	11	1.03	1.14	6000
12	29	F	124	86	69	52	54.5	45.5	1.20	1.06	9	9	1.05	1.13	134
13	24	F	128	80	84	58	59.4	40.6	1.46		13	10	1.04	1.11	156
14	38	F	126	82	69	43	74.5	25.5	2.92	0.59	11	6	1.07	1.08	6000
15	28	F	138	80	70	67	72.4	27.6	2.62	0.92	9	11	1.18	1.16	154
16	26	M	120	86	71	58	64.5	35.5	1.82	0.75	11	12	1.14	1.12	164
17	38	M	132	86	76	65	67	33	2.03	0.69	13	8	1.1	1.12	164
18	39	M	128	80	83	52	56.4	43.6	1.29	0.79	7	9	1.06	1.19	6000
19	38	M	130	80	76	53	62.2	37.8	1.65	0.78	9	10	1.04	1.13	2348
20	32	M	128	86	72	64	69.4	30.6	2.27	0.96	9	8	1.08	1.17	120
21	34	M	132	80	81	39	70.4	29.6	2.38	1.09	11	11	1.09	1.12	119
22	36	M	130	80	70	38	64.5	35.5	1.82	0.86	7	10	1.12	1.05	136
23	35	M	130	80	75	64	65.3	34.7	1.88	0.87	11	8	1.08	1.12	214
24	28	M	128	82	78	36	62.8	37.2	1.69	1.05	9	9	1.06	1.02	154
25	23	M	130	82	65	34	64.3	35.7	1.80	0.76	12	10	1.01	1.01	544
26	25	M	132	80	74	54	75.3	24.7	3.05	0.68	14	7	1.12	1.1	6000
27	36	M	130	84	72	47	68.4	31.6	2.16	1.06	11	8	1.18	1.02	198
28	27	M	124	80	79	54	66.3	33.7	1.97	0.85	9	10	1.14	1.03	6000
29	26	M	126	80	69	37	72.5	27.5	2.64	1.12	7	8	1.08	1.05	124
	38	M	136	80	74	46	66.8	33.2	2.01	0.83	12	11	1.09	1.03	190